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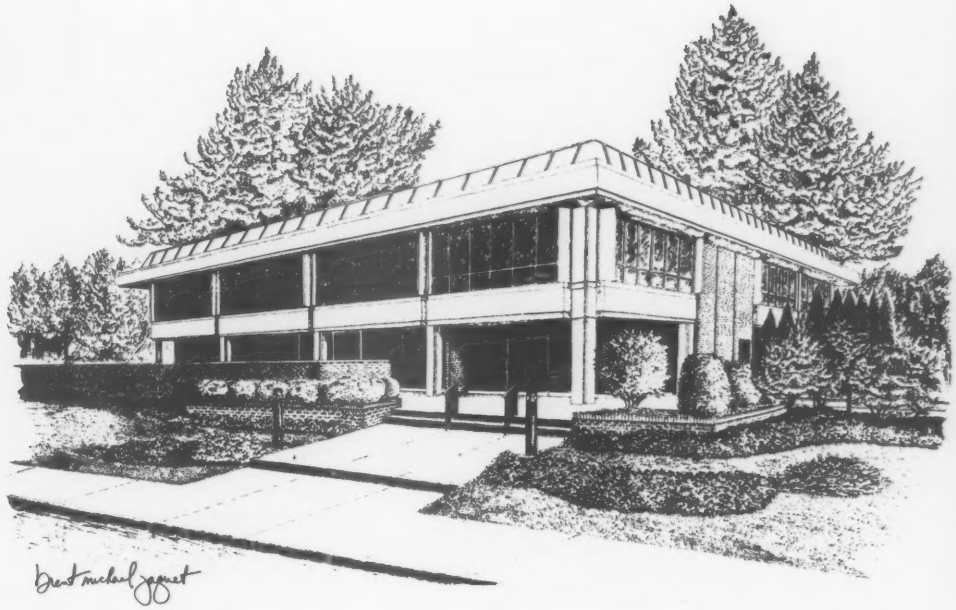

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Local Treatment of Ewing's Sarcoma: Radiation Therapy Versus Surgery[1]

Timothy J. Kinsella,* Allen S. Lichter, James Miser, Lynn Gerber, and Eli Glatstein[2]

In his initial histological description of diffuse endothelioma of bone in adolescents and young adults, Ewing (1) noted that these tumors were highly radioresponsive following the now antiquated treatment with radium molds. He described the typical gross appearance of the permeative tumorous destruction of bone with an associated soft tissue swelling representing tumor infiltration of adjacent muscles and spread along fascial planes. Complete tumor regression was reported following irradiation with later remodeling of normal bone matrix and return of normal limb function. Throughout the orthovoltage era and now well into the megavoltage era of radiation therapy, the efficacy of local radiation therapy in Ewing's sarcoma has been repeatedly confirmed (2-7). The excellent local control achieved in primary, often bulky, tumors has even led some centers to consider total-body irradiation as a systemic adjuvant in patients with Ewing's sarcoma (8-10).

Recently, it has been suggested that radical surgery with either amputation or wide excision be considered as an alternative to radiation therapy in the local management of Ewing's sarcoma (11,12). These investigators cite the potential problems of local tumor recurrence, normal tissue injury to irradiated bone and soft tissues, and the risk of second tumors following the use of high-dose radiation therapy. Other authors have suggested the deferral of initial local treatment to assess the response of the primary to systemic chemotherapy (13-15). Indeed, if a complete response is achieved by the use of chemotherapy, then the subsequent use of local radiation therapy has been questioned (12,15).

In view of these controversies regarding the local treatment of Ewing's sarcoma, a reassessment of the role of radiation therapy seems timely. In this article, we address the issues pertinent to the choice of local treatment in Ewing's sarcoma. We closely examine issues of local control and normal tissue injury following high-dose local irradiation and compare these results to the more limited data available on surgical and/or chemotherapeutic approaches. Our recommendations on radiation technique, total radiation dose, and volume are outlined. Finally, we discuss options for the integration of local irradiation and systemic treatment for patients with Ewing's sarcoma.

Is Local Therapy Necessary?

Most authorities would agree that Ewing's sarcoma is fundamentally a systemic disease. Indeed, up to one-third of patients with Ewing's sarcoma will have evidence of metastatic disease in lung or other bones at presentation following a thorough staging work-up (16,17). The percentage of patients with overt metastases at presentation is much greater among patients with a primary tumor of the proximal extremity or central axis (up to 50%) than among those with a distal extremity primary (approximately 10%) (17). However, even in patients with localized disease, the most common pattern of failure is with metastatic disease, often within the first 2 years from diagnosis, despite intensive multiagent chemotherapy (9,18-20).

Given that Ewing's sarcoma is a systemic disease in most patients, one might argue that local treatment may be less important than systemic therapy and should not compromise the delivery of systemic therapy. The impact of local therapy on disease-free survival in patients with localized Ewing's sarcoma is somewhat difficult to analyze. Prior to the routine use of chemotherapy, Phillips and Higinbotham (21) at Memorial Hospital reported that 25% of patients with "localized" Ewing's sarcoma were long-term survivors following irradiation to the entire affected bone. The term "localized" is used advisedly since these patients were staged prior to the availability of routine lung tomography, computerized tomographic (CT) scanning, and radionuclide bone scanning. Although some of these long-term survivors did receive a short course of single-agent chemotherapy, any benefit from this adjuvant therapy is difficult to ascertain. Shortly thereafter a study by the European Organization for Research on Treatment of Cancer, comparing local irradiation to local irradiation plus low-dose alkylating chemotherapy, showed no difference in either local control or disease-free survival with the addition of low-dose single-

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agent chemotherapy (22). Again, 25% of patients with "localized" disease were cured with radiation therapy alone.

With the use of adjuvant combination chemotherapy, it appears that the survival rates for patients with localized Ewing's sarcoma are considerably better. The improvement in disease-free survival is most impressive in patients with a distal extremity primary, where 60%–70% may be cured (disease-free survival > 2–3 years) (14,16,17). However, < 35% of patients with primary tumors of the proximal extremity or pelvis are long-term disease-free survivors (10,17,18). Further, for patients who present with metastatic disease, combination chemotherapy has not had a major impact on curability (16,17). Many of these patients have not been rendered disease-free with systemic therapy and only a rare patient presenting with distant metastases has been a long-term survivor (17). In fact, patients presenting with metastatic disease are not included among many analyses of multimodal therapy (4,18). Many groups are advocating the use of local irradiation to metastatic sites in these patients with poor prognosis and there appears to be some improvement in the complete response rates with this combined modality approach for such patients (10,18). Thus, even in the era of aggressive multidisciplinary treatment, the location of the primary tumor and the presence of metastases at initial staging remain the major prognostic variables in Ewing's sarcoma.

Despite the influence of adjuvant combination chemotherapy on disease-free survival, it is unclear whether there has been any additive effect of chemotherapy to local irradiation in terms of local control. In a review of the National Cancer Institute (NCI) experience using standard local irradiation (5000 rad/5 weeks to the entire bone) and progressively more aggressive chemotherapy, no significant difference in clinical local control was found with the addition of doxorubicin or dactinomycin to the two-drug regimen of vincristine and cyclophosphamide (6). Our results on local control are comparable to other clinical series using high-dose irradiation (ie, 5000–6000 rad), showing that < 10% of patients with distal lesions will experience local failure compared to 20%–40% of patients with high proximal extremity or central (usually pelvic) lesions (18–20).

It must be kept in mind that an analysis of local control in Ewing's sarcoma is particularly difficult for two reasons. First, it is a disease which commonly metastasizes to bone, and there is a possibility of metastatic tumor reseeding a previously sterilized primary site. Although local tumor reseeding is speculative, it may in part explain the difference in clinical and pathological local control rates. That reseeding could occur is evidenced by the typically rapid (a few weeks to a few months) development of widespread metastatic disease to lung, pleura, and bone seen in a relapsed Ewing's patient. It is not surprising that in the terminal Ewing's patient with extensive bony metastatic disease there is clinical and/or pathological evidence of tumor at the initial irradiation site (as is seen at multiple other bony sites). In the small but often-quoted autopsy series from the NCI, approximately two-thirds (13 of 20 evaluated patients, all with known disseminated neoplasms) showed histologic evidence of malignant cells within the primary site (23). The clinical relevance of these data on local control is unclear, since these patients typically died from widely metastatic disease and often manifested no clinical evidence of local failure prior to death. Within the NCI experience, local recurrence as the initial site of relapse remains very uncommon (6,10).

A second major problem in assessing local control following high-dose irradiation is the interpretation of follow-up diagnostic studies including x-rays, CT scans, and radionuclide scans. Typically, there is prompt tumor regression during the first 3–4 weeks of irradiation associated with a decrease in pain, tenderness, and local erythema. However, many patients with bulky soft tissue masses (usually in the pelvis) have a considerably smaller but persistent mass seen on follow-up CT scanning for many months after completion of treatment. Additionally, diagnostic studies of the irradiated bone (x-rays and bone scans) may often continue to show abnormal results for months to years following treatment. These abnormalities can be a source of concern and frustration to the clinician, particularly in patients with no obvious metastatic disease. The differential diagnosis includes persistent tumor, a smoldering osteomyelitis, radiation fibrosis of bone and soft tissues, and least likely, an evolving radiation-induced second tumor. Unfortunately, the medical literature does not detail how best to manage such a patient. Our general approach is *not* to biopsy the soft tissue or bone routinely because of the high probability of poor wound healing and/or infection as well as the difficulty of interpreting the limited pathological material obtained by needle biopsy or a small open biopsy. Patients in this situation are closely followed (every 4 weeks) and, if local symptoms or signs worsen, then a careful open biopsy of the soft tissue abnormality is performed to provide adequate material for light and electron microscopy as well as for microbiological studies.

Given (*a*) the clinical local failure rate of 10% in distal extremity lesions and up to 35% for central lesions following high-dose radiation therapy, (*b*) the potential problem of local tumor reseeding from metastatic sites, and (*c*) the diagnostic dilemmas of assessing soft tissue and bony abnormalities following treatment, it seems reasonable to re-examine the role of primary surgical management of Ewing's sarcoma. Although strong opinions have been raised to support the role of surgery (11,12), the major source of long-term data is a retrospective review of 194 patients with Ewing's sarcoma treated at the Mayo Clinic between 1912 and 1968 (24). During that period of time, there were substantial changes in the

techniques of surgery and radiation therapy, as well as important changes in clinical staging, which were not addressed in this retrospective review. Indeed, the criteria for amputation were not outlined and the authors admit that the radiation dose and tumor volume were so variable that any analysis of the results of radiation therapy would be meaningless. Nevertheless, the claim was made that patients undergoing surgery alone or surgery as part of their treatment showed a survival advantage over nonsurgical treatment (including those patients with central disease and/or with overt metastases), with follow-up for up to 30 years. Yet, this survival advantage appears confined to patients with distal extremity lesions (with amputation, approximately 30% of patients were long-term survivors). Certainly, these survival data are *not* different from series using high-dose radiation therapy alone on comparable patients (ie, those with localized distal extremity lesions) prior to the era of chemotherapy (21,22). Additionally, the authors furnish no data analyzing local control following amputation, which may be an important issue with high proximal extremity primaries.

The traditional surgical approach to primary bone tumors is an amputation of the entire affected bone. Thus, for a lesion of the distal extremity, an above-elbow or above-knee amputation might be performed, while a disarticulation would be required for a lesion of the humerus or femur. In Ewing's sarcoma, where the soft tissue extension often crosses the joint space proximally, a more aggressive surgical approach, such as high above-knee amputation, hemipelvectomy, or forequarter amputation, would have to be considered for such lesions. More recently, limb-sparing procedures have gained some popularity in the treatment of primary bone tumors. The surgery involves a wide (en bloc) resection of the primary tumor with insertion of either a bone allograft or more commonly a metallic implant. Unfortunately, the applicability of this approach to Ewing's sarcoma is limited both by the extensive extraosseous soft tissue spread and by the intraosseous medullary cavity spread, both of which are characteristic of this tumor. Additionally, there is also a possibility of clinically undetectable "skip" lesions in the affected bone which would not be removed by the surgical procedure. The exact frequency of "skip" lesions in Ewing's sarcoma is not known but may be higher than the reported figure of 20% for osteogenic sarcoma (25). The assumption is that systemic treatment will completely eradicate any such intraosseous tumor deposits. Ideally, a limb-sparing approach is suited for adolescents or older patients who have attained normal or near normal growth and who have small tumors which do not involve adjacent major nerves or vessels and are not associated with significant soft tissue extensions. This kind of surgery has generally been used for select lesions about the knee (26,27), although with a small proximal humeral lesion, an en bloc upper humeral intrascapulothoracic resection (Tikhoff-Linberg procedure) may be used (28).

A comparison of local control following surgery or radiation therapy is complicated by the selection factors (frequently unspecified) used in judging patients suitable for such surgery. As previously stated, the large clinical series from the Mayo Clinic did not attempt to analyze local control following amputation (24). Theoretically, if the amputation is performed one joint above the gross tumor, then local control should be excellent. However, in the presence of a soft tissue extension, which can be massive in many patients with high proximal extremity and pelvic tumors, even the most radical surgical procedure would not be locally curative. Additionally, patients who present with metastatic disease are seldom considered for an ablative surgical procedure since their prognosis is so dismal even with aggressive systemic therapy (16,17). These poor-risk patients are treated with local irradiation to the primary and/or metastatic sites, often with persistent local control in the setting of progressive metastatic disease (10).

There is a recent trend toward the use of preoperative chemotherapy to reduce tumor bulk followed by wide excision in extremity, rib, and selected pelvic lesions (12,15). Typically, three to five cycles of combination chemotherapy are administered prior to consideration for wide excision. Postoperative radiation therapy is reserved only for patients with gross or microscopic disease documented at surgery. "Moderate-dose" irradiation in the range of 3500–4000 rad is used on the assumption that with the combined chemotherapy and surgical debulking, less irradiation is required. Whether this assumption is true remains to be seen. However, in the two clinical series combining preoperative chemotherapy and wide excision, approximately 60% (29 of 50 patients) required postoperative irradiation. The follow-up of these patient groups is too short (< 2 years) to assess local control adequately. However, it appears that even with a good response to combination chemotherapy (often difficult to assess for bony lesions of any type), in the vast majority of these selected patients surgery will reveal residual disease, often gross, that necessitates further local treatment. Paradoxically, postoperative irradiation often requires the use of larger radiation fields to cover surgically manipulated tissues adequately. Additionally, any metallic implant can cause problems with radiation dosimetry. Finally, no objective analysis of functional outcome after "limited" resection is available regarding even these selected patient groups.

Another potential limitation to primary surgical management of Ewing's sarcoma is indirectly realized in the Mayo Clinic review where no patient with a central axis primary received surgery alone (24). For example, there are obvious limitations to surgery in patients with a vertebral primary which is often associated with extradural spinal cord or cauda equina compression. Additionally, in a primary tumor of the ilium abutting a sacroiliac joint,

there are enormous difficulties in establishing negative surgical margins, even with an extended hemipelvectomy. The more recent use of CT scanning has often documented soft tissue extension from the primary bone tumor which in retrospect was clearly not appreciated using only physical examination combined with conventional plain and contrast x-ray studies; for such patients, the CT scan now allows accurate definition of the extent of tumor for the first time. In many patients with a pelvic primary, gross soft tissue extension into the abdomen and/or retroperitoneum may be such that even the most aggressive curative surgical approach is not technically feasible.

The poorer local control rates (65%–80%) reported for the proximal extremity and pelvic lesions following high-dose radiation therapy are probably primarily related to the tumor bulk. However, these data antedate the routine use of CT scanners, and gross underestimation of tumor volume may, in part, be responsible for the increased risk of local failure for such lesions (fig 1). In our most recent series of patients with proximal extremity and central axis primaries who routinely had CT staging, local failure has occurred in only 12% of patients (three of 24; with a *minimum* follow-up of 2 years), which is approximately one-half our previous local failure in similar groups of patients (9). Obviously, longer follow-up and more patients are needed to assess any improvement in local control of central axis Ewing's sarcoma attributed to CT-directed high-dose radiation therapy.

Complications of Local Therapy

In any discussion comparing surgery versus radiation therapy as local treatment, an integral part of the analysis should involve treatment complications and morbidities. With the use of radiation therapy, both acute and late effects need to be considered realizing that most patients will be receiving concomitant or sequential chemotherapy, including dactinomycin and/or doxorubicin. If one assumes a low operative mortality (< 1%) at major cancer centers for these young patients, the major complications of surgical treatment will be late effects, although acute morbidity secondary to infection or prosthesis breakage may occur. Obviously, any significant rate of postoperative infection also implies delay of scheduled chemotherapy with potential compromise of systemic management of this typically rapidly growing neoplasm.

The acute effects of irradiation are related to location

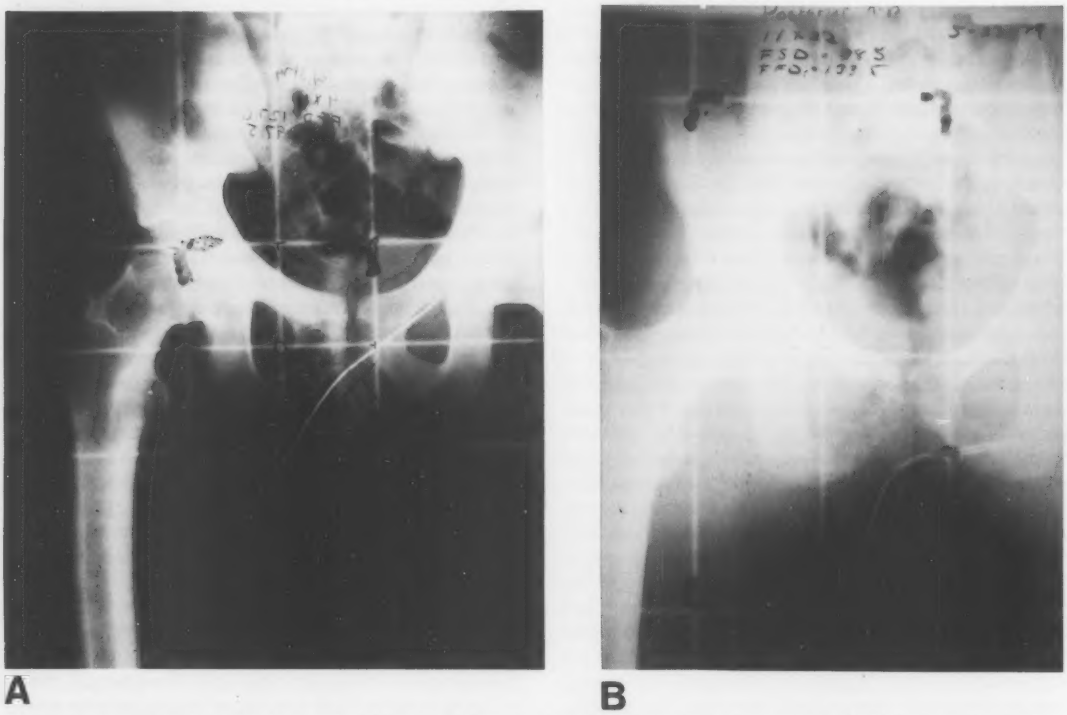

FIGURE 1.—Comparison of the planned radiation field based on information from physical examination, bone scanning, and plain x-rays (A) and following CT scanning (B). The CT scan documented extensive soft tissue tumor infiltration into the pelvis not evident by other techniques. These films are taken on a simulator; rectangles highlighted by crayon markers on the corners represent the size of the treatment portals (A) initially and (B) finally planned on the same patient with a Ewing's sarcoma arising from the pubis.

of the primary tumor, volume of normal tissue(s) included within the radiation fields, total dose, and dose rate. These effects are typically expressed in normal rapidly dividing tissues (skin, gut, and bone marrow) and are felt to represent a disruption of the normal homeostatic self-renewal ("stem cell") cell population. As such, these effects are reversible and can be modified by lowering the dose rate. Late effects are more worrisome, often progressive (irreversible), and probably result from damage to the normal supporting stroma of a tissue or organ. The volume of normal tissue(s) irradiated and total dose are the major variables of late effects. Late effects may not be expressed for months to years following treatment and are not necessarily predicted by the severity of the acute effects.

Both acute and late effects of irradiation can be reduced by the use of careful and proper irradiation technique. As a general rule, the entire affected bone and well-defined soft tissue extension are defined as the tumor volume. Since Ewing's sarcoma is known to infiltrate widely into normal tissues, at least a 3-5-cm margin is included in the treatment fields around the gross tumor evident by CT scanning. Patients should be routinely simulated to optimize treatment planning. With extremity lesions, the use of casts to immobilize the extremity during treatment is particularly helpful. CT scanning in the treatment position often results in further sparing of normal tissues by the use of customized blocks and usually facilitates the sparing of uninvolved normal tissues by utilizing isocentric oblique fields rather than opposing anterior-posterior fields. Patients are treated using 175-200-rad daily fractions to a total dose of 5000 rad over 5-6 weeks. With bulky lesions (> 10 cm), we routinely boost the residual soft tissue and bone abnormality (based on repeat CT scanning after 5000 rad) for an additional 1000 rad.

Acute radiation effects in treating extremity lesions are primarily manifest as skin changes (erythema progressing to moist or dry desquamation) during or shortly following therapy. Generally, these skin effects resolve within 5-15 days of stopping radiation therapy, although confluent moist desquamation may recur following chemotherapy with dactinomycin or doxorubicin. To minimize the risk of this drug-radiation toxicity, a planned radiation treatment break (4-5 days) at the time of chemotherapy is often helpful. As stated previously, acute radiation effects are rarely a source of major morbidity.

On the other hand, late radiation effects in the treatment of extremity lesions may range from minimal subcutaneous fibrosis to severe fibrosis with muscle contractures. Similarly, the irradiated bone may show long-term effects ranging from mild osteopenia to marked cortical defects with a pathological fracture. In the available limited literature, there appear to be considerable differences in the frequency of these severe late effects. In a review of 29 patients with Ewing's sarcoma of the lower extremity treated at the NCI with high-dose irradiation and combination chemotherapy, only one patient (< 4%) had a functional deficit that was severe enough to warrant amputation (29). Over one-half of the patients had essentially normal leg function, with a minimum follow-up of 2 years. Moreover, our more recent experience with careful treatment planning using CT scans with the patient in the *exact* treatment position appears to be yielding further improvements in the long-term functional results. These results are in direct contrast to a series from Memorial Hospital, where up to one-half of the patients had posttreatment functional deficits comparable to the deficit of an amputation (30). The radiation techniques in that series differed from the NCI approach in volume, dose, and methods of treatment.

In addition to proper radiation technique, the explanation for these discrepancies in lower extremity function following high-dose irradiation may reflect the age of the patient, type of biopsy, and routine use of physical rehabilitation. In the prepubertal patient, long-bone irradiation results in growth arrest in the epiphyseal plate. To minimize the growth difference between the irradiated and unirradiated leg, it is often possible to block the epiphyseal plate at the opposite end from the bone primary. Fortunately, many patients with Ewing's sarcoma will have completed or almost completed growth at the time of diagnosis. Additionally, we recommend that only a small incisional biopsy of bone or preferably of just the soft tissue be performed to establish the diagnosis. The cortical bone defect should be avoided if possible or at least minimized to limit the subsequent risk of pathological fracture, especially with large weight-bearing bones. Indeed, the risk of fracture after treatment may well reflect the biopsy process as much as neoplasm or treatment. Finally, we strongly advocate the implementation of a regular program of active and passive exercise including range of motion and strengthening during irradiation and for long-term maintenance following irradiation. It is helpful to have an experienced physician or physical therapist screen the patient prior to treatment to assess accurately the functional deficit related to the tumor and to devise a treatment plan to optimize muscle and joint function. Skin care should also be stressed with avoidance of excessive thermal or physical stress to the irradiated area during and following therapy. We believe careful physical therapy to be important in optimizing function in these patients and grossly underemphasized in the literature.

For the upper extremity, including the hand, the proper use of high-dose irradiation typically results in only a minimal functional deficit (31,32). Functional outcome for patients receiving radiation therapy is clearly superior to amputation in upper extremity lesions. The radiation treatment and follow-up recommendations outlined for the lower extremity are directly applicable to primary

tumors of the arm, forearm, and hand. Additionally, in radiation treatment planning of the distal forearm, hand, or foot, where the tissue thickness may be ≤ 6 cm, we employ a treatment technique using a water bath with complete immersion of the involved distal extremity to improve the radiation dose distribution of the modern high-energy x-ray beams (31). Following the initial 3000-4000 rad using the water bath, a moderate skin reaction necessitates a short treatment break (5-7 days) after which bolus material to the gross tumor site replaces the water bath.

In general, we feel that with careful attention to fine details of radiation treatment planning, the functional deficits following high-dose irradiation to the upper and lower extremity are quite acceptable (29,31). However, it must be kept in mind that some patients may not be suitable for definitive radiation therapy. There may be individual patients presenting with significant pretreatment functional deficits related to infiltration of nerves and vessels who may actually be better suited to an amputation. In the patient with extensive destruction of a weight-bearing bone (often associated with a displaced pathological fracture), the treatment options include amputation or an attempt at limb preservation with a bone prosthesis. In the latter situation, we have integrated surgery, chemotherapy, and radiation therapy to optimize the chance of local control in one patient. The involved extremity was placed in a bivalve cast which was removed daily for preoperative irradiation. Following adequate regression of the soft tissue mass (after 3500-4000 rad and chemotherapy), the tumorous bone and residual soft tissue tumor were resected and the prosthesis was placed. Following wound healing, we used an additional 1500-2000 rad for histologically positive margins and continued chemotherapy.

Following surgery, the functional outcome is determined by the level of amputation, subsequent growth, risks of infection, type of prosthetic appliance, and patient compliance to the use of the prosthesis (33). Surprisingly, little objective information is available on extremity function following amputation in adolescents, even for osteosarcoma, where amputation is commonly used (34,35). In general, the patient is mobilized 24 hours after a lower extremity amputation and rehabilitation is started. Often, a plastic, temporary prosthesis is used for the first few weeks with the definitive prosthesis being fitted at 4-6 weeks following the surgery. In the growing adolescent, subsequent bone spurring at the amputated end may result in severe pain and soft tissue swelling, which can restrict mobility or require surgical reversion. Additionally, the use of chemotherapy often results in a chronic loss of soft tissue about the stump which can impair prosthetic fitting and rehabilitation. With below-knee and some above-knee prostheses, function can be almost normal following rehabilitation. However, with a high above-knee amputation prosthesis, or particularly an upper extremity prosthesis, functional deficits remain even with successful rehabilitation and patients are often not prosthesis users.

The long-term functional results following limb-sparing surgery are not clearly defined. Significant short-term complications have been realized with resection of the distal femur secondary to infection, prosthesis breakage, chronic joint effusion, and occasionally instability requiring bracing or tumor recurrence. In one series of patients with osteosarcoma, up to one-third developed complications eventually requiring an amputation (26). Knee-joint function may be impaired by the endoprosthesis (26,36). In the upper humerus, the Tikhoff-Linberg procedure results in good hand and forearm function which provides a distinct functional advantage over a shoulder disarticulation or forequarter amputation. Outside of this procedure, it is not evident whether the long-term functional results of limb-sparing procedures are any better than amputation and rehabilitation. The time required before patients return to a reasonable functional level is often quite protracted (up to 1 year) in those undergoing limb-sparing procedures.

Finally, the issue of second tumors arising in patients successfully treated for primary Ewing's sarcoma needs to be discussed. Of 16 cases of second tumors reported in the Ewing's sarcoma literature, 11 cases of bone sarcomas (mostly osteosarcoma) occurring within the radiation field have been described (37-39). Although it appears that these adolescent patients are definitely at risk to develop bone sarcomas in the irradiated area, the exact frequency cannot be accurately determined from the small population groups analyzed to date (38,39). Whether the combination of radiation therapy and chemotherapy is more carcinogenic in these patients is also not clear (38,39). Furthermore, it is possible that some patients initially diagnosed as having Ewing's sarcoma may actually have the small cell variant of osteosarcoma initially, which responds to irradiation but later recurs and is mistakenly scored as a second tumor (40,41). There are no data to establish that patients with Ewing's sarcoma are "cancer-prone" nor do these patients have severe acute radiation effects to normal tissues as described for some patients with retinoblastoma or ataxia telangiectasia (42,43). Although the induction of second tumors is obviously of major concern, we do not feel that the available data are by any means compelling enough to not recommend radiation therapy in the treatment of Ewing's sarcoma.

CONCLUSIONS

Although Ewing's sarcoma is a systemic disease with progressive metastatic disease still representing the most common pattern of treatment failure, local treatment of the primary site (and possible metastatic sites) remains an important aspect of curative treatment. An analysis

of our experience at the NCI, from the viewpoints of both local control and function, supports the use of primary radiation therapy in most patients with Ewing's sarcoma. In selected patients, staging by a multidisciplinary team of radiation and surgical oncologists, physiatrists, and possibly orthopedic surgeons is essential for identifying patients with high risk of morbidity from either amputation or irradiation. After risk factors are identified, an appropriate treatment plan can be selected that optimizes local control and function. We feel that the technique of radiation therapy and the use of an appropriate rehabilitation program, which includes range of motion exercise, skin care, and education about appropriate physical activity, are important aspects of successful local treatment.

REFERENCES

1. EWING J. Diffuse endothelioma of bone. Proc NY Pathol Soc 21:17-24, 1921.
2. WANG CC, and SCHULTZ MD. Ewing's sarcoma. N Engl J Med 248:571-576, 1953.
3. PHILLIPS RF, and HIGINBOTHAM NL. The curability of Ewing's endothelioma of bone in children. J Pediatr 70:391-397, 1967.
4. HUSTU HO, PINKEL D, and PRATT CB. Treatment of clinically localized Ewing's sarcoma with radiotherapy and combination chemotherapy. Cancer 30:1522-1527, 1972.
5. JOHNSON RF, and POMEROY TC. Evaluation of therapeutic results in Ewing's sarcoma. AJR 123:583-587, 1975.
6. TEPPER J, GLAUBIGER D, LICHTER A, ET AL. Local control of Ewing's sarcoma of bone with radiotherapy and combination chemotherapy. Cancer 46:1969-1973, 1980.
7. KINSELLA TJ, TRICHE TJ, DICKMAN PS, ET AL. Extraskeletal Ewing's sarcoma: results of combined modality treatment. J Clin Oncol 1:489-495, 1983.
8. JENKIN RDT, RIDER WD, and SONLEY MJ. Ewing's sarcoma: adjuvant total body irradiation, cyclophosphamide and vincristine. Int J Radiat Oncol Biol Phys 1:407-413, 1976.
9. KINSELLA TJ, MITCHELL JB, MCPHERSON SJ, ET AL. In vitro radiation studies on Ewing's sarcoma cell lines and human bone marrow: implications for the clinical use of total body irradiation. Int J Radiat Oncol Biol Phys 8(Suppl 1):93, 1982.
10. KINSELLA TJ, GLAUBIGER D, DEISSEROTH A, ET AL. Intensive combined modality therapy including low dose TBI in high risk Ewing's sarcoma patients. Int J Radiat Oncol Biol Phys 9:1955-1960, 1983.
11. PRITCHARD DJ. Indications for surgical treatment of localized Ewing's sarcoma of bone. Clin Orthop 153:39-43, 1980.
12. MARCOVE RC, and ROSEN G. Radical en bloc excision of Ewing's sarcoma. Clin Orthop 153:86-91, 1980.
13. ROSEN G, CAPARROS B, MOSENDE C, ET AL. Curability of Ewing's sarcoma and consideration for future therapeutic trials. Cancer 41:888-899, 1978.
14. ROSEN G, CAPARROS B, NIRENBERG A, ET AL. Ewing's sarcoma: ten-year experience with adjuvant chemotherapy. Cancer 47:2204-2213, 1981.
15. HAYES FA, THOMPSON EI, HUSTU HO, ET AL. The response of Ewing's sarcoma to sequential cyclophosphamide and adriamycin induction therapy. J Clin Oncol 1:45-51, 1983.
16. GEHAN EA, NESBIT ME, BURGERT EO, ET AL. Prognostic factors in Ewing's sarcoma. Natl Cancer Inst Monogr 56:273-278, 1981.
17. GLAUBIGER DL, MAKUCH RW, and SCHWARTZ J. Influence of prognostic factors on survival in Ewing's sarcoma. Natl Cancer Inst Monogr 56:285-288, 1981.
18. TEFFT M, RAZEK A, PEREZ C, ET AL. Local control and survival related to radiation dose and volume and to chemotherapy in non-metastatic Ewing's sarcoma of pelvic bones. Int J Radiat Oncol Biol Phys 4:367-372, 1978.
19. GASPARINI M, LOMBARDI F, GIANNI C, ET AL. Localized Ewing's sarcoma: results of integrated therapy and analysis of failures. Eur J Cancer 17:1205-1209, 1981.
20. PEREZ CA, TEFFT M, NESBIT M, ET AL. The role of radiation therapy in the management of non-metastatic Ewing's sarcoma of bone. Report of the intergroup Ewing's sarcoma study. Int J Radiat Oncol Biol Phys 7:141-149, 1981.
21. PHILLIPS RE, and HIGINBOTHAM NL. The curability of Ewing's endothelioma of bone in children. J Pediatr 70:391-397, 1967.
22. ZUCKER JM, and HENRY-AMAR M. Therapeutic controlled trial in Ewing's sarcoma. Eur J Cancer 13:1019-1023, 1977.
23. TELLES NC, RABSON AS, and POMEROY TC. Ewing's sarcoma: an autopsy study. Cancer 41:2321-2329, 1978.
24. PRITCHARD DJ, DAHLIN DC, DAUPHINE RT, ET AL. Ewing's sarcoma: a clinicopathological and statistical analysis of patients surviving five years or longer. J Bone Joint Surg 57:10-16, 1975.
25. ENNEKING WF, and KAGAN A. "Skip" metastases in osteosarcoma. Cancer 36:2192-2205, 1975.
26. MARCOVE RC. En bloc resections for osteogenic sarcoma. Cancer Treat Rep 62:225-231, 1978.
27. SIM FH, IVINS JC, and PRITCHARD DG. Surgical treatment of osteogenic sarcoma at the Mayo Clinic. Cancer Treat Rep 62:205-211, 1978.
28. MARCOVE RC, LEWIS RJ, and HUVOS AG. En block upper humeral intrascapulo-thoracic resection—the Tikhoff-Linberg procedure. Clin Orthop 124:21-26, 1977.
29. JENTZSCH K, BINDER H, CRAMER H, ET AL. Leg function after radiotherapy for Ewing's sarcoma. Cancer 47:1267-1278, 1981.
30. LEWIS RJ, MARCOVE RC, and ROSEN G. Ewing's sarcoma—functional effects of radiation therapy. J Bone Joint Surg 59:325-331, 1977.
31. KINSELLA TJ, LOEFFLER JS, FRAASS BA, ET AL. Extremity preservation by combined modality therapy in sarcomas of the hand and foot: an analysis of local control disease-free survival and functional result. Int J Radiat Oncol Biol Phys 9:1115-1119, 1983.
32. KLIMAN M, HARWOOD AR, JENKIN RD, ET AL. Radical radiotherapy as primary treatment for Ewing's sarcoma distal to the elbow and knee. Clin Orthop 165:233-238, 1982.
33. KEGEL B, CARPENTER ML, and BURGESS EA. A survey of lower limb amputees: prostheses, phantom sensations and psychological aspects. Bull Prosthet Res 1:43-60, 1977.
34. ENNEKING WF, and SPRINGFIELD DS. Osteosarcoma. Orthop Clin North Am 8:785-803, 1977.
35. THORPE W, GERBER LH, LAMBERT M, ET AL. A prospective study of rehabilitation of the above knee amputee with rigid dressings. Clin Orthop 143:133-137, 1979.
36. MARCOVE RC, LEWIS MM, ROSEN G, ET AL. Total femur replacement. Comb Ther 3:13-18, 1977.
37. LI FP, CASSADY JR, and JAFFE N. Risk of second tumors in survivors of childhood cancer. Cancer 35:1230-1235, 1975.
38. STRONG LC, HERSON J, OSBORNE BM, ET AL. Risk of radiation-related subsequent malignant tumors in survivors of Ewing's sarcoma. JNCI 62:1401-1406, 1979.
39. GREENE MH, GLAUBIGER DL, MEAD GD, ET AL. Subsequent cancer in patients with Ewing's sarcoma. Cancer Treat Rep 63:2043-2046, 1979.
40. SIM FH, UNNI KK, BEABOUT JW, ET AL. Osteosarcoma with small cells simulating Ewing's tumor. J Bone Joint Surg 61:207-215, 1979.
41. MARTIN SE, DWYER A, KISSANE JM, ET AL. Small cell osteosarcoma. Cancer 50:990-996, 1982.
42. SAGERMAN RH, CASSADY JR, TRETTER P, ET AL. Radiation-induced neoplasia following external beam therapy for children with retinoblastoma. AJR 105:529-535, 1969.
43. CUNLIFFE PN, MANN JR, CAMERON AH, ET AL. Radiosensitization in ataxia telangiectasia. Br J Radiol 48:374-376, 1975.

The Management of Ewing's Sarcoma: Role of Radiotherapy in Local Tumor Control[1]

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The role of radiotherapy in the management of Ewing's sarcoma is discussed in view of both historical and current treatment policies. In particular, a comparison of radiotherapy and surgery as modalities for local control is presented. The technical aspects of dose and volume of radiotherapy are discussed together with its time relationship to chemotherapy and surgery. Although studies are in progress evaluating lower doses and smaller volumes than those used traditionally, until the results of these are available it would seem prudent to recommend the standard therapy as outlined in the guidelines of the recent Intergroup Ewing's Sarcoma Study Trial (IESS-II). Recommended doses are: 4500 rad to the whole bone (except the contralateral epiphysis when the tumor is at or near the end of a long bone) plus a 5-cm margin around the tumor and any soft tissue extension, followed by 500 rad to a 5-cm margin, followed by 500 rad to a 1-cm margin given at 180–200 rad/day 5 days/week. Since many studies have shown it to be safe and possibly advantageous to postpone irradiation until induction chemotherapy has been completed, this appears to be the emerging approach. The value of partial excision (debulking) prior to irradiation is unclear at the present time. Radiotherapy may not be indicated for those patients with tumors of the lower limb when the unfused epiphyses would need to be irradiated. In addition, patients with pathologic fractures and with tumors of bones that are deemed expendable (such as ribs or clavicles) are probably best managed by complete surgical resection. [Cancer Treat Rep 68:703–710, 1984]

Over the past 10 years great advances have taken place in the management of patients with Ewing's sarcoma, with substantial improvements both in local tumor control and long-term survival.

These improved results are directly related to (*a*) better staging of patients through more sophisticated diagnostic procedures, (*b*) more precise surgical and radiotherapeutic approaches to eradication of the primary tumor, and (*c*) more rational and aggressive chemotherapy for control of disseminated micrometastases.

As advances are made through improved therapy, attention should be turned toward a more stringent assessment of therapy toxicity. Therefore, it is important to consider (*a*) selection of patients who can be treated with a limited resection, without anatomical or functional deficit, in contrast to those patients who will require irradiation, (*b*) use of a combination of moderate doses of irradiation to limited volumes of bone, combined with resection of the gross tumor, (*c*) technical factors that will affect results from irradiation, and (*d*) sequelae of irradiation, in terms of both anatomical disfiguration or functional disturbances and probability of developing a second malignant tumor within the radiation field.

This paper will review existing data from the literature and will offer suggestions for appropriate application of irradiation in the treatment of Ewing's sarcoma.

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HISTORICAL CONSIDERATIONS

Although in 1949 Geschickter and Copeland (1) recommended surgical resection including, where necessary, amputation as the principal treatment modality in Ewing's sarcoma, they commented that shrinkage of the tumor after two or three radiation treatments was characteristic of the disease. They also pointed out that irradiation prolonged life as well as alleviated symptoms.

In 1953 Wang and Schulz (2) reported that six of 36 patients treated by definitive radical irradiation compared with one of 14 patients treated by resection lived 5 years. They concluded that external irradiation was the treatment of choice. Reviews of the literature by Tefft et al (3) and Perez et al (4) compared the experience before and after the era of intensive chemotherapy and showed that not only did the 5-year survival increase with chemotherapy but the local control rate was also enhanced from approximately 65%-75% to about 90%-95%. Table 1 shows the incidence of local control after radiotherapy in many series (4).

Further reports from the M. D. Anderson Hospital (5), Memorial Hospital (6), and the Intergroup Ewing's Sarcoma Study (IESS) (7) have confirmed that the combination of radiotherapy and chemotherapy gives excellent local control rates and that the main obstacle to higher survival rates remains the development of distant metastases.

However, many problems other than distant metastases still exist. Although local control rates are good, the morbidity of combined irradiation and chemotherapy is substantial, particularly in the long bones of young children, and in some cases is greater than that of surgery plus chemotherapy. In addition, the problem of second malignancies is beginning to be appreciated as patients survive long enough to develop them. The local control rates reported for combined modality therapy by clinical evaluation may also be higher than those actually achieved, as in one series (8) which demonstrated persistent residual tumor at the primary site in 13 of 20 patients examined pathologically at the time of autopsy.

The implications of these observations in the overall management of the disease are discussed in the ensuing sections.

RADIOTHERAPY VERSUS SURGERY

The two issues in this controversy concern local control, which influences survival, and late normal tissue effects. The local control following an amputation with an adequate margin is obvious, and so are the late effects. Less clear is the likelihood of local control by irradiation in any particular situation or the late effects that can be expected in each case. The issues are not entirely resolved, but certain guidelines can be developed from analysis of the accumulated experience.

TABLE 1.—Incidence of local control of primary lesion in nonmetastatic Ewing's sarcoma (*from* ref 4)

Dose of irradiation (rad)	Chemotherapy		Duration of follow-up	No. of patients	Local control (%)	Institution
	Drugs*	Duration				
3000-5000	—	—	5 yrs	6	3 (50)	Roswell Park
4000-5000	CYC	1 yr	3 yrs	6	3 (50)	Roswell Park
4000-6000	—	—	5 yrs	25	14 (56)	M. D. Anderson
3700-4400	—	—	5 yrs	6	4 (67)	Memorial Hospital
4000	—	—	5 yrs	7	5 (71)	University of California at San Francisco
4500	—	—	5 yrs	11	8 (73)	University of California at San Francisco
4000-5000	CYC + VCR + ADR + AMD	1½ yrs	3 yrs (median)	24	20 (83)	IESS
5000-6000	CYC + VCR + AMD ± ADR	1½ yrs	37 mos (median)	51	44 (86)	IESS
6000-7400	—	—	5 yrs	15	10 (67)	M. D. Anderson
6000-8000	—	—	5 yrs	8	6 (75)	Memorial Hospital
6000-7500	CYC + VCR + AMD + ADR	2 yrs	12-24 mos	16	14 (88)	Memorial Hospital
4700-6300	CYC + VCR	1-2 yrs	4-91 mos	15	14 (93)	St. Jude Hospital
6000	CYC + VCR	34 wks	14-46 mos	19	18 (95)	M. D. Anderson
6000-7000	CYC + VCR + AMD ± ADR	1½ yrs	31 mos (median)	20	20 (100)	IESS

* CYC = cyclophosphamide; VCR = vincristine; ADR = doxorubicin; and AMD = dactinomycin.

Survival

Analysis of 194 patients with nonmetastatic tumors at diagnosis treated at the Mayo Clinic (9) showed that when surgery (other than biopsy) was part of the treatment (70 patients) the 5-year survival rate was 34.3%, whereas when no surgery was performed (124 patients) the 5-year survival rate was 10.5% ($P = 0.0001$). Analyzing only patients with tumors of the extremities, who had a better prognosis than those with trunk primaries independent of treatment, 47 patients whose treatment included surgery (usually amputation) had a 5-year survival rate of 44.7% as opposed to 13.1% in 61 patients treated without surgical resection ($P < 0.002$). The choice of modalities for treatment of the primaries was left to the investigators' choice.

In the first trials reported by the IESS (IESS-I), patients with primary nonmetastatic disease were not subject to any randomization involving surgery, but surgery was again left to the investigators' choice. Eight of 57 patients treated surgically died, whereas 81 of 205 died without primary surgical treatment. Median survival was 240 weeks for patients treated with surgery and 152 weeks for the others ($P = 0.01$) (10).

However, a recent unpublished analysis of this same study has shown that prognosis depends also on the size of the lesion (L. A. Gilula and M. A. Foulkes, personal communication). Patients with lesions < 10 cm in greatest diameter have a statistically significantly longer disease-free survival than those who have larger tumors. Experience from patients with rib primaries in IESS-I strongly suggested that the good prognosis in the patients treated surgically is related to patient selection, with smaller tumors more likely to be resected (11).

Late Effects

Although amputation clearly produces profound disability in most instances, there are situations in which irradiation will produce equal or greater sequelae. For instance, irradiation of the foot frequently produces considerable underdevelopment and pain, whereas loss of part or all of the foot and replacement with a prosthesis are compatible with almost normal function. In other instances, such as certain tumors of the fibula, ribs, etc, amputation is not necessary and wide excision of the tumor and involved bone results in little or no disability.

Irradiation of the unfused epiphyseal plates will cause shortening of the affected limb and, in the case of the trunk, additional asymmetry. Moreover, soft tissue changes, pathologic fractures, and second malignancy in the irradiated sites may ensue. Since chemotherapy is always part of the therapy for children with Ewing's sarcoma, increased acute and late effects are to be expected compared with the same dose of irradiation alone (12).

After reviewing the experience at Memorial Hospital, Lewis et al (13) concluded that surgery should be considered for patients with lesions of the lower extremity (in particular, the foot or distal femur) and for those with pathologic fractures. However, the experience at the National Cancer Institute suggested that when the dose is limited to 5000 rad and when supervoltage irradiation with shaped fields is used along with chemotherapy, the functional results are favorable (14).

IESS Late Effects Study

Preliminary results from the IESS-I late effects study do reveal substantial secondary effects in some patients; at this time they have not been related to treatment regimen (J. R. Neff and M. E. Nesbit, unpublished communication). An analysis of the data available on patients surviving 2 years off treatment suggests that the secondary effects are most profound in the weight-bearing portion of the skeleton.

Limb length inequalities in the upper extremities appeared well-tolerated and in most instances were of little clinical significance. Postirradiation fibrosis and stiffness were also well-tolerated and usually were compatible with satisfactory function. Edema distal to the treatment area, although unusual with the treatment techniques recommended, appeared most problematic in view of the dependent position of the upper extremity.

Primary lesions involving the vertebra were usually associated with shortening and asymmetry. This was, however, well-tolerated. Underdevelopment of the mandible, as well as the need for dental extraction, was noted in one patient with a cervical primary lesion. Those patients with reported secondary effects and thoracic and lumbar primaries developed shortening of 2–4 inches from their projected height. One patient developed sufficient asymmetry to warrant management with a Milwaukee brace.

The expected localized postirradiation fibrosis of the lung in those patients with primary rib lesions was in general well-tolerated. No functional restrictions were reported.

Primary Ewing's sarcoma of the hemipelvis was commonly associated with late effects in patients surviving their illness. A significant limp, as well as stiffness, was associated with primary lesions in the ilium. These lesions tended to be larger and associated with more bone destruction. Limb length inequality was not as significant as that reported with those lesions involving the distal femur and proximal tibia (areas where significant growth disturbance might be expected from altered growth induced by treatment of the distal femoral and proximal tibial epiphyses).

Primary lesions of the femur yielded the most significant secondary effects. Two-thirds of those patients with abnormalities developed shortening > 2 cm, with one-third developing pathologic fractures. Three patients required amputation for reasons other than pathologic

fracture (fibrosis, shortening, stiffness, etc).

Shortening associated with primary tibial lesions occurred less frequently than that with femoral lesions. Of those patients with reported secondary effects, one in four required amputation for pathologic fracture and/or a nonfunctional painful extremity. Treatment of primary lesions of the fibula did result in some shortening, usually treated with a shoelift. One patient developed asymmetric growth requiring corrective osteotomy. There were no reported amputations for late effects of primary lesions of the fibula.

In general, lesions of the hands, feet, and digits did not tolerate radiotherapy well.

A review of these preliminary data suggests significant orthopedic complications in 25% of patients. Certainly, many of these effects can be managed either with orthoses or operative procedures.

Second Malignancy

Although it is well-known that children cured of one cancer are at risk of developing another (15), a series from the the M. D. Anderson Hospital highlighted the problem for survivors of Ewing's sarcoma (16). Four new bone tumors occurred in heavily irradiated areas in 24 patients followed 3-22 years for Ewing's sarcoma, whereas 1.2×10^{-3} tumors were expected. Three of these patients also had received intensive chemotherapy. However, these patients received either orthovoltage to 4000-5500 rad or megavoltage to 6000-7000 rad. The median dose was 6100 rad in patients developing second tumors.

Of 251 irradiated patients in IESS-I, only three (1.2%) have been reported to have developed second malignancies; all three were osteosarcomas within the radiotherapy portals (J. R. Neff and M. E. Nesbit, unpublished communication). Two patients with pelvic primary lesions and one with primary lesion of the fibula have been reported. All three died of osteosarcoma.

Although there may possibly be some under-reporting in the IESS figures, the discrepancy between these and the M. D. Anderson experience is perhaps more likely to be dose-related. In IESS-I, only 67 of 251 patients (27%) received doses ≥ 6000 rad (7).

Recommendations

In general, therefore, surgical treatment is recommended for lower-extremity lesions in patients with unfused epiphyses and for patients who have, or are very likely to develop, pathologic fractures. It is also recommended, where possible, to excise "expendable" bones, such as ribs, clavicles, fibulae, etc.

RADIOTHERAPY TECHNIQUES

Radiotherapeutic principles dictate that a tumor be irradiated with a wide margin (dimensions dependent on anatomy and histology) to eradicate undetected microscopic spread of disease. Gross tumor will require higher doses than microscopic foci (17).

Dosage

As mentioned earlier in this article, the addition of chemotherapy to radiotherapy has increased not only the disease-free survival but also the local control rate. The results from IESS-I (7) showed that a local tumor control of approximately 90% could be achieved but no dose reponse could be demonstrated (table 2). Accordingly, since the complication rate was dose-dependent, the recommended dose in the successor studies (IESS-II) has been 4500 rad to the whole bone with two boosts of 500 rad each to a tumor margin of 5 cm and then 1 cm. Preliminary analysis of the latter study has shown no decreased local tumor control but it is too early for any definitive statement. At St. Jude Hospital, an attempt has been made to reduce the tumor dose to 3000-3500 rad following induction chemotherapy (18). Four of 19 patients had experienced local recurrence (22%) with a median follow-

TABLE 2.—IESS-I: correlation of dose of irradiation and local tumor control*

Tumor dose (rad)	Pelvis (including sacrum)			Femur, humerus			Tibia, fibula			Other		
	I	II	III	I	II	III	I	II	III	I	II	III
3000-3999	2/2	1/1	1/1	1/1	0/0	0/0	1/1	0/0	0/0	3/3	1/1	1/1
4000-4999	7/7	2/3	1/1	6/6	3/6	3/4	4/4	2/2	4/4	11/13	1/1	5/5
5000-5999	10/14	7/7	11/15	14/16	10/12	16/17	7/7	6/6	7/8	13/13	8/9	5/6
≥ 6000	3/3	2/2	3/3	10/10	7/9	10/10	8/8	8/9	6/8	5/5	4/5	2/2
Totals	22/26	12/13	16/20	31/33	20/27	29/31	20/20	16/17	17/20	32/34	14/16	13/14
	50/59 (84.7%)			80/91 (87.9%)			53/57 (92.9%)			59/64 (92.1%)		
	242/271 (89.3%)											

*Values = No. of patients achieving local tumor control/No. receiving dose. Regimen I = VCR, AMD, CYC, and ADR; regimen II = VCR, AMD, and CYC; and regimen III = VCR, AMD, CYC, and bilateral pulmonary irradiation.

up of 20 months (E. I. Thompson, personal communication). Therefore, until more information is available we would recommend the dose of 4500 rad to a wide field, with a 500–1000-rad boost. Irradiation should be given at 900–1000 rad/week (180–200 rad in five daily fractions) in most instances. Doses per fraction in excess of the above are likely to produce considerable morbidity due in part to the additive effect of chemotherapy.

Volume

Classical teaching held that the tumor spread through the entire bone and into surrounding soft tissues and lymphatics and that the irradiation fields should be extended to include all these tissues (19). However, Suit (20) pointed out the lack of recurrences in portions of the bone not clinically or radiologically involved at the time of the initiation of treatment provided that the entire bone was irradiated. He therefore suggested that, since chemotherapy was effective, it could possibly substitute in part and thereby allow the irradiation dose to the whole bone to be reduced.

In IESS-I the initial treatment volume included the whole bone; there was to be a boost to the gross radiographic tumor with associated soft tissue mass (6). The results showed that < 10% of patients with adequate treatment volume had local relapse, in contrast to 29% with minor field variation (portion of the epiphysis not included) or to 23% with major variances in field size (entire bone not irradiated or boost dose delivered to volume with margin < 5 cm around the gross residual tumor). These differences were, however, not statistically significant ($P > 0.05$), and the local recurrences, where recorded accurately, were found to be at the sites of the initial bulky disease and never at the site of the uninvolved epiphysis. It was also noted in this study that the site of disease was important. For example, pelvic disease had a 13% local recurrence rate, whereas for fibula it was 3% (table 2).

It is of interest that in IESS-I no increased local failures were noted in the patients treated with the four-drug (including ADR) combination if the dose or volume was "inadequate." This was in contrast to the observation in the patients who received VCR, AMD, and CYC only, for whom, even though a dose response was not observed, "inadequate portals" resulted in a higher incidence of local failures (table 3) (24).

For IESS-II, it was therefore decided that, when the tumor was at or near the end of a long bone, the contralateral epiphysis would not be included in the treatment portals (fig 1). This would reduce late effects, particularly growth disturbances. Preliminary results of IESS-II have not shown recurrences in the uninvolved epiphysis. Treatment portals in other respects were not changed over the guidelines for IESS-I.

In the St. Jude study (18), where chemotherapy was given as induction therapy for 12 weeks (five courses) prior to radiotherapy, the radiation portals were confined to the original bone destruction and any soft tissue mass present at the end of the induction. Although there were four local recurrences, all of these were in the center of the original tumor rather than at the margin or outside the port (E. I. Thompson, personal communication).

In an attempt to reduce the complication rate, the Pediatric Oncology Group is beginning a protocol of "tailored" radiotherapy versus standard radiotherapy after induction chemotherapy. The dose of radiation will be the same in both arms: 4000 rad plus 1500-rad boost. The tailored volume will include treatment to the area of the involved bone and associated original soft tissue extension with a 2-cm margin, whereas the standard treatment portals will include the whole bone in addition.

The Childrens Cancer Study Group is also considering a tailored volume for its study. There would, however, be no radiotherapy randomization.

Unless a patient is in a study, we would recommend the volumes used in IESS-II. However, judicious shielding of adjacent normal uninvolved tissues is vital. Computed tomography can be very useful in planning such treatment. Wherever possible, when long bones are to be treated, a margin of skin and subcutaneous tissues should be spared to allow lymphatic drainage.

Timing of Radiotherapy and Chemotherapy

Ewing's sarcoma is usually highly symptomatic, with

TABLE 3.—Local control and dose and volume of irradiation correlated with chemotherapy regimen: all sites (*from* ref 21)*

	Adequate volume		Inadequate volume (major)		Inadequate volume (minor)	
Tumor dose (rad)	Regimen I	Regimens II–III	Regimen I	Regimens II–III	Regimen I	Regimens II–III
3000–3999	6/6	3/3	1/1	1/1	0/0	0/0
4000–4999	23/24	15/16	4/5	3/4	1/1	3/6
5000–5999	30/34	58/65	11/12	5/7	3/4	7/8
⩾ 6000	21/21	35/38	2/2	1/2	3/3	6/8
Totals	80/85 (94%)	111/122 (91%)	18/20 (90%)	10/14 (71.4%)	7/8 (87.5%)	16/22 (72.7%)

* Values = No. of patients achieving local tumor control/No. receiving dose. Chemotherapy regimens are defined in footnote to table 2.

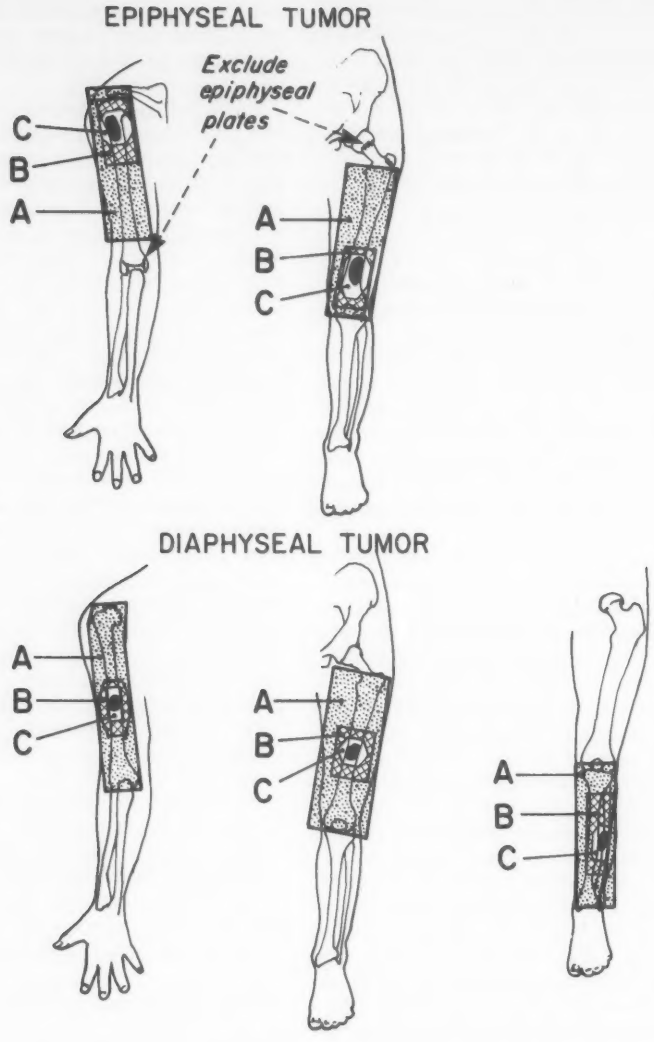

FIGURE 1.—Examples of treatment portals used in IESS-II. When the eccentric location of the tumor allows it, one of the epiphyseal centers is spared to diminish growth disturbances. A = entire bone; B = tumor with 5-cm margins; and C = portal used for boost to the residual primary tumor.

pain being a presenting complaint. Therefore, in the prechemotherapy era it was essential that irradiation be started as soon as possible. This was also the case in the early trials, such as IESS-I, where at the start of the trial the value of chemotherapy had been suggested but not proven.

The results of IESS-I showed that distant metastases, which often appeared early, were the major problem. Although the local relapse rate was 12% (7), distant metastases occurred in 52.3% (22). The distant metastasis rate was particularly high in patients with pelvic and sacral primaries. It was also noted that reduction of the time interval between chemotherapy courses from 6 to 3 weeks reduced the relapse rate (22).

In addition, chemotherapy was frequently noted to eliminate or reduce symptoms rapidly and to promote

healing of an eroded bone at the start of irradiation. It was therefore proposed that, in patients where the prognosis was poor and where a significant proportion of bone marrow was to be irradiated, radiotherapy should be postponed until a high dose of chemotherapy had been given. Thus, the patients with pelvic and sacral primaries on IESS-II received 6 weeks of high-dose intermittent chemotherapy before proceeding to surgery and/or radiotherapy. Preliminary results suggest a superior relapse-free survival and longer time to first metastasis in patients receiving IESS-II (M. A. Foulkes and E. A. Gehan, personal communication).

The St. Jude Hospital protocol involves a 12-week "induction" therapy with sequential CYC and ADR. This is followed by radiotherapy and/or surgery (18). Since 15 of 19 patients with localized disease were in complete remission at the end of induction (E. I. Thompson, personal communication), this approach seems highly encouraging. Although there were four local recurrences noted, these may be dose-related (3000–3500 rad).

At Memorial Hospital, the practice for many years has been to postpone radiotherapy or surgery until after two or three courses of chemotherapy have been given (6).

Both the Pediatric Oncology Group and Childrens Cancer Study Group proposed studies employing initial chemotherapy and delayed irradiation. Therefore, this must now be considered the standard therapeutic approach. However, since excessive delay could possibly lead to reduced local control, these studies must be monitored very carefully for this potential problem.

Debulking Surgery

A smaller tumor is more radiocurable than a larger one, with all other parameters being equal. Therefore, in theory at least, a judicious combination of both surgery and radiotherapy might be able first to avoid the problems caused by surgery as radical as amputation or by high-dose large-volume irradiation and second to improve the tumor control rate.

Although no studies have been performed randomizing for surgical treatment, the analysis of the rib primaries in IESS-I suggested that patients with complete excision (with or without radiotherapy) did better than those with partial excision and irradiation (11). However, seven of eight tumors completely excised measured < 10 cm in greatest diameter compared with four of nine in the debulked patients.

The use of debulking surgery, therefore, remains to be evaluated; "debulking chemotherapy" may, however, be more practical.

CONCLUSIONS

(*a*) Radiotherapy in combination with multiagent chemotherapy controls a large proportion of primary tumors in patients with Ewing's sarcoma. This is closely related to the size of the lesion.

(*b*) Increased local control of the primary lesion by irradiation in conjunction with systemic chemotherapy results in higher survival rates.

(*c*) At the present time, irradiation doses of 4500 rad to a large volume involving the primary tumor, including soft tissues, with a boost of 500–1000 rad to reduced fields, seem to be adequate. Lower doses will require further investigation.

(*d*) Tailored irradiation portals, to spare uninvolved normal tissues, result in decreased morbidity.

(*e*) In lesions located in bones that can be resected without severe anatomical functional deficit, a surgical approach should be preferred to irradiation.

(*f*) It may be advantageous to delay irradiation until after initial chemotherapy.

(*g*) Even though great progress has been made in the treatment of patients with Ewing's sarcoma and both local control and survival have been raised significantly over the past 20 years, further clinical trials will be necessary since a large proportion of the patients still die because of distant metastases.

REFERENCES

1. GESCHICKTER C, and COPELAND M. Ewing's sarcoma. *In* Tumors of Bone, 3rd ed. 1949, pp 387–434.
2. WANG CC, and SCHULZ MD. Ewing's sarcoma. N Engl J Med 248:571–576, 1953.
3. TEFFT M, CHABORA BM, and ROSEN G. Radiation in bone sarcomas. Cancer 39:806–816, 1977.
4. PEREZ CA, RAZEK AA, TEFFT M, ET AL. Analysis of local control in Ewing's sarcoma. Cancer 40:2864–2873, 1977.
5. CHAN RC, SUTOW WW, LINDBERG RD, ET AL. Management and results of localized Ewing's sarcoma. Cancer 43:1001–1006, 1979.
6. ROSEN G, CAPARROS B, NIRENBERG A, ET AL. Ewing's sarcoma: ten-year experience with adjuvant chemotherapy. Cancer 47:2204–2213, 1981.
7. PEREZ CA, TEFFT M, NESBIT ME, ET AL. Radiation therapy in the multimodal management of Ewing's sarcoma of bone: report of the Intergroup Ewing's Sarcoma Study. Natl Cancer Inst Monogr 56:263–271, 1981.
8. TELLES NC, RABSON AS, and POMEROY TC. Ewing's sarcoma: an autopsy study. Cancer 41:2321–2329, 1978.
9. PRITCHARD DJ, DAHLIN DC, DAUPHINE RT, ET AL. Ewing's sarcoma. J Bone Joint Surg 57-A:10–16, 1975.
10. PRITCHARD DJ. Surgical experience in the management of Ewing's sarcoma of bone. Natl Cancer Inst Monogr 56:169–171, 1981.
11. THOMAS PRM, FOULKES MA, GILULA LA, ET AL. Primary Ewing's sarcoma of the ribs. Cancer 51:1021–1027, 1983.
12. TEFFT M, LATTIN PB, JEREB B, ET AL. Acute and late effects on normal tissues following combined chemo- and radiotherapy for childhood rhabdomyosarcoma and Ewing's sarcoma. Cancer 37:1201–1213, 1976.
13. LEWIS RJ, MARCOVE RC, and ROSEN G. Ewing's sarcoma: functional effects of radiation therapy. J Bone Joint Surg 59:324–331, 1977.
14. JENTZSCH K, BINDER H, CRAMER H, ET AL. Leg function after radiotherapy for Ewing's sarcoma. Cancer 47:1267–1278, 1981.
15. LI P, CASSADY JR, and JAFFE N. Risk of second tumors in survivors of childhood cancer. Cancer 35:1230–1235, 1975.
16. STRONG LC, HERSON J, OSBORNE BM, ET AL. Risk of radiation-relat-

ed subsequent malignant tumors in survivors of Ewing's sarcoma. JNCI 62:1401-1408, 1979.
17. FLETCHER GH. Textbook of Radiotherapy, 3rd ed. Lea & Febiger, 1980.
18. HAYES FA, THOMPSON EI, HUSTU HO, ET AL. The response of Ewing's sarcoma to sequential cyclophosphamide and adriamycin induction therapy. J Clin Oncol 1:45-51, 1983.
19. HUSTU HO, PINKEL D, and PRATT CB. Treatment of clinically localized Ewing's sarcoma with radiotherapy and combination chemotherapy. Cancer 30:1522-1527, 1972.
20. SUIT HD. Role of therapeutic radiology in cancer of the bone. Cancer 35:930-935, 1975.
21. PEREZ CA, TEFFT M, NESBIT ME, ET AL. The role of radiation therapy in the management of non-metastatic Ewing's sarcoma of bone. Report of the Intergroup Ewing's Sarcoma Study. Int J Radiat Oncol Biol Phys 7:141-149, 1981.
22. NESBIT ME, PEREZ CA, TEFFT M, ET AL. Multimodal therapy for the management of primary nonmetastatic, Ewing's sarcoma of bone: an intergroup study. Natl Cancer Inst Mongr 56:255-262, 1981.

Preliminary Results of High-Dose Busulfan and Cyclophosphamide With Syngeneic or Autologous Bone Marrow Rescue[1,2]

Calvin Lu, Hayden G. Braine,* Herbert Kaizer, Rein Saral, Peter J. Tutschka, and George W. Santos[3,4]

The toxic effects of high-dose busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg) with autologous or syngeneic bone marrow rescue were evaluated in 19 patients (11 with acute myelocytic leukemia, one with acute lymphocytic leukemia, one with acute myelofibrosis, two with chronic myelocytic leukemia, one with Hodgkin's disease, and three with non-Hodgkin's lymphoma). Their mean age was 26 years (range, 6–50); nine patients had syngeneic and ten had autologous bone marrow rescue (six of whom had in vitro bone marrow incubation with 4-hydroperoxycyclophosphamide). Severe myelosuppression was expected and was seen in all patients; leukocyte and platelet count recovery occurred at a median of 19 days (range, 11–59) and 30 days (range, 20–89), respectively. Nausea, vomiting, and diarrhea were frequent but readily managed with vigorous medical therapy. Stomatitis was severe in 14 patients. Skin, renal, cardiac, pulmonary, and CNS complications directly attributable to drug-related toxic effects were transient and non-life-threatening. Hepatic function abnormalities were common but tended to be transient. Most patients tolerated high-dose busulfan and cyclophosphamide with manageable side effects. Hepatic veno-occlusive disease was fatal in two patients, while diffuse interstitial pneumonitis with disseminated herpes virus infection was fatal in three patients with lymphoma. All patients treated in relapse or without previous therapy had a complete tumor response. Further studies with this regimen should be pursued. [Cancer Treat Rep 68:711–717, 1984]

Busulfan is an alkylating agent in which the biologic activity is associated with aromatic sulfonic acid ester groups. Over the past 30 years, busulfan has been used primarily in chronic myelocytic leukemia (CML) and polycythemia rubra vera, usually in low daily oral doses. In addition to bone marrow depression (1), reported side effects at these doses have included skin hyperpigmentation (1), nausea (2), vomiting (2), diarrhea (2), amenorrhea (3), an Addison's-like wasting syndrome (4), and interstitial pulmonary fibrosis (5). A number of other side effects have been even less commonly reported (6–16), and their association with busulfan has not always been clearly delineated. Clinical trials with daily oral low-dose busulfan in other malignancies have usually resulted in excessive myelosuppression, with very low response rates (17). Used at higher intermittent doses, busulfan has resulted in a significant response rate (18–21), but severe or even irreversible myelosuppression has limited further trials with this agent.

At The Johns Hopkins Oncology Center, high-dose busulfan with cyclophosphamide have been used since 1978 as a preparative regimen for allogeneic bone marrow transplantation in patients with acute leukemia. Experience with this regimen has shown a significant antileukemic effect (22). Evaluation of drug-related

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toxicity, however, has been confounded by the presence of concurrent graft-versus-host disease following allogeneic transplantation. Therefore, in this study, we have limited our evaluation of the toxic effects of high-dose busulfan and cyclophosphamide to patients with acute leukemia, CML, and lymphoma who received autologous or syngeneic bone marrow rescue. This has allowed us the opportunity to examine the toxicity of this regimen in the absence of graft-versus-leukemia effect and graft-versus-host disease.

MATERIALS AND METHODS

Patient Characteristics

The clinical course of 19 consecutive patients with lymphohematologic malignancies treated with busulfan (4 mg/kg/day orally on Days 1-4) and cyclophosphamide (50 mg/kg/day iv on Days 5-8) followed by autologous or syngeneic bone marrow rescue at The Johns Hopkins Oncology Center between September 1978 and April 1982 was reviewed, with follow-up to October 1, 1982. The age, sex, type of bone marrow rescue, and previous chemotherapy of the 11 patients with acute myelocytic leukemia (AML), one with acute lymphocytic leukemia (ALL), one with acute myelofibrosis (AMF), two with CML, and four with lymphoma (one with Hodgkin's disease and three with non-Hodgkin's lymphoma [NHL]) are shown in table 1. Of the 11 patients with AML, four were in first remission (Unique Patient Number [UPN] 137, 162, 267, and 316), two were in second remission (UPN 258 and 314), two were in third remission (UPN 309 and 344), one was in sixth remission (UPN 294), one was in early first relapse (UPN 342), and one was in early second relapse (UPN 212). The patient with ALL was in early second relapse. One patient with CML was in chronic phase (UPN 249), while the other was in blastic phase (UPN 209). The histologic subtypes of the patients with NHL included nodular poorly differentiated lymphocytic (UPN 158), diffuse histiocytic (UPN 178), and undifferentiated (UPN 224) lymphomas. All patients were considered to have an extremely poor prognosis with conventional therapy.

TABLE 1.—Patient characteristics

UPN	Age (yrs)	Sex	Diagnosis	Type of bone marrow rescue (4-HC)*	Previous therapy†	Response‡	Outcome
137	10	M	AML	Syngeneic	Ara-C, Drn	—	Relapsed on Day 326
158	36	F	NHL	Syngeneic	Adr, Chl + RT	CR	Died on Day 13 in remission
162	24	M	AML	Syngeneic	Ara-C, Prd, TG, Vcr	—	In remission on Day 1234
178	35	F	NHL	Autologous	Adr, Ara-C, Bleo, Cy, Prd, Vcr, VP-16 + RT	CR	Died on Day 60
179	18	M	HD§	Autologous	Adr, BCNU, Bleo, Vbl + RT	CR	Died on Day 24 in remission
209	30	M	CML	Autologous	Cy, Dex	CR	Died on Day 41 in remission
212	12	M	AML	Syngeneic	Adr, Ara-C, Aza, Cy, Prd, TG, Vcr	CR	Relapsed on Day 75
224	18	F	NHL	Autologous	Bleo, Vcr + RT + Adr, Cy	CR	Relapsed on Day 91
249	19	F	CML	Syngeneic	Bu	CR	Relapsed on Day 482
251	50	M	AMF	Syngeneic	None	CR	Relapsed on Day 81 (CNS), Day 156 (bone marrow)
258	6	F	AML	Autologous (60)	Adr, Ara-C, Dex, Drn, Prd, TG, Vcr	—	Relapsed on Day 145
267	10	M	AML	Autologous (60)	Adr, Ara-C, Aza, MP, Mtx, Prd, Vcr	—	In remission on Day 581
286	18	M	ALL	Syngeneic	Adr, Ara-C, Aza, Hdu, MP, Mtx, Prd, Vcr	CR	Relapsed on Day 114
294	43	M	AML	Autologous (80)	Ara-C, Cy, Drn, *m*-AMSA, MP, Mtx, VP-16	—	Died on Day 36 in remission
309	45	M	AML	Autologous (80)	Adr, Ara-C, Drn, Prd, TG, Vcr	—	In remission on Day 360
314	30	M	AML	Autologous (80)	Ara-C, Drn, Prd, TG, Vcr	—	Relapsed on Day 194
316	44	F	AML	Syngeneic	Adr, Ara-C, Prd, Vcr	—	In remission on Day 339
342	38	M	AML	Syngeneic	Adr, Ara-C, Prd, Vcr	CR	In remission on Day 175
344	19	M	AML	Autologous (100)	Ara-C, Cy, Dex, Drn, *m*-AMSA, MP, Mtx, Prd, TG, Vcr, VP-16 + RT	—	In remission on Day 158

*4-HC = 4-hydroperoxycyclophosphamide; values in parentheses = bone marrow treated in vitro with 4-HC in μg/ml.

†Ara-C = cytarabine; Drn = daunorubicin; Adr = doxorubicin; Chl = chlorambucil; RT = radiation therapy; Prd = prednisone; TG = 6-thioguanine; Vcr = vincristine; Bleo = bleomycin; Cy = cyclophosphamide; VP-16 = etoposide; BCNU = carmustine; Vbl = vinblastine; Dex = dexamethasone; Aza = 5-azacitidine; Bu = busulfan; MP = 6-mercaptopurine; Mtx = methotrexate; Hdu = hydroxyurea; and *m*-AMSA = amsacrine.

‡If treated in relapse or without previous therapy; CR = complete remission.

§HD = Hodgkin's disease.

Bone Marrow Harvest

Bone marrow cells were collected by multiple bone marrow aspirations under general anesthesia and processed, as previously described (23). In syngeneic transplantation, the marrow cells were promptly infused into the recipient without further processing. In autologous bone marrow rescue, the marrow cells were cryopreserved, as has been previously described (24). Six of the ten autologous transplantation patients were part of a study (25) in which their marrow cells were incubated in vitro before freezing with 4-hydroperoxycyclophosphamide at concentrations of 40–100 μg/ml at 37°C for 30 minutes, washed twice, resuspended in autologous plasma, and then frozen. At the time of marrow rescue, the frozen cells were transported to the bedside in a portable liquid nitrogen tank, thawed rapidly in a water bath at 37°C, and infused iv immediately.

Bone Marrow Transplantation

The patients were hospitalized in single-bed rooms which received 28 nonlaminar air exchanges/hour through 0.30-μm ceiling air filters, with reverse isolation procedures (handwashing and masks). On Day -14 (with Day 0 as the day of marrow infusion), a diagnostic lumbar puncture was performed, followed by instillation of intrathecal methotrexate (10 mg/m^2; maximum, 12 mg) or cytarabine (30 mg/m^2; maximum, 40 mg). On Day -9, the patients received busulfan (4 mg/kg/day orally) in four divided doses for 4 consecutive days (total dose, 16 mg/kg) in gelatin capsules containing up to eight 2-mg tablets. On Day -5, the patients received cyclophosphamide (50 mg/kg iv) over 30 minutes on 4 consecutive days (total dose, 200 mg/kg), while a diuresis of 100 ml/hour was maintained, with the use of diuretics and antiemetics as needed. On Day 0, marrow was infused via an iv line without a filter.

The patients subsequently received standardized parenteral hyperalimentation, appropriate fluid and electrolyte supplementation, irradiated blood component support, prophylactic oral nonabsorbable antibiotics, and antibiotic therapy as required. Diuretics were used liberally to prevent fluid retention and the development of noncardiogenic pulmonary edema.

RESULTS

Myelotoxicity

All of the patients developed severe myelosuppression, with profound granulocytopenia (polymorphonuclear count < 100/mm^3) and thrombocytopenia (platelet count < 20,000/mm^3), as well as a rbc transfusion requirement (uncorrected reticulocyte count < 1%) (fig 1). At the time of initiation of high-dose busulfan and cyclophosphamide, three patients still had granulocytopenia and thrombocytopenia from previous systemic chemotherapy.

Granulocytopenia developed in the other 16 patients at a median of 2 days (range, -2 to 4) following bone marrow infusion. Granulocyte recovery (polymorphonuclear count > 500/mm^3) occurred in 16 of 19 patients

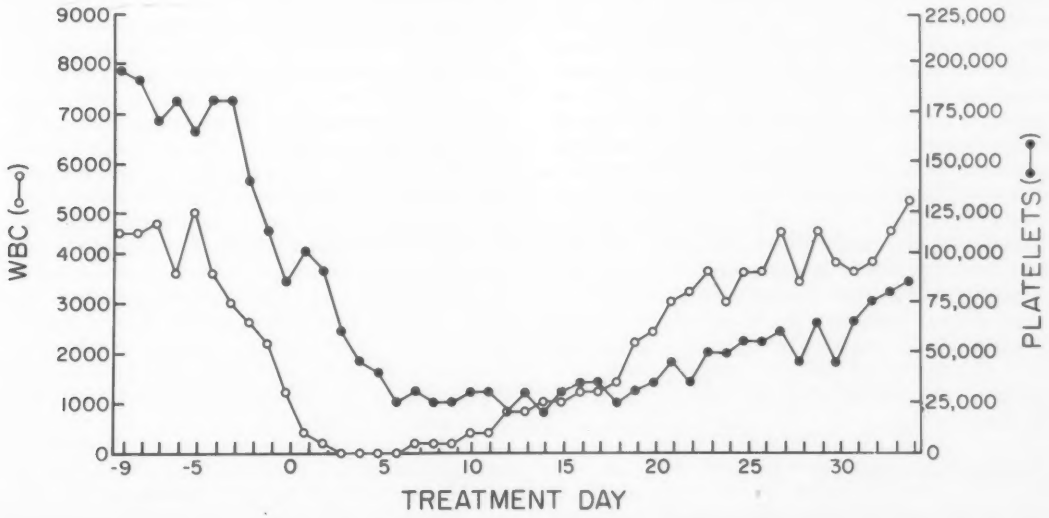

FIGURE 1.—Mean total wbc and platelet counts, as related to treatment day. Bone marrow infusion given on Day 0.

(including all three patients who initially had granulocytopenia) at a median of 19 days (range, 11-59) following bone marrow infusion. Median duration of granulocytopenia in the 13 evaluable patients was 19 days (range, 10-57). All three patients who died prior to granulocyte recovery had evidence for early bone marrow engraftment at autopsy.

Thrombocytopenia developed in the 16 patients who did not already have thrombocytopenia at a median of 4 days (range, -1 to 7) following bone marrow infusion. Thereafter, the platelet count was supported to a level > 20,000/mm^3 with platelet transfusions. Platelet count recovery (> 50,000/mm^3) lagged behind granulocyte recovery, occurring in 14 of 19 patients (including two of three patients who initially had thrombocytopenia) at a median of 30 days (range, 20-89) following bone marrow infusion, with a median duration of thrombocytopenia of 27 days (range, 16-89) in the 12 evaluable patients. All five patients who died prior to platelet count recovery had either peripheral hematologic recovery in at least one other cell line or evidence at autopsy of early bone marrow engraftment.

Reticulocyte count recovery (uncorrected count > 2%) occurred in 15 of 19 patients at a median of 25 days (range, 17-73). Again, the four remaining patients had evidence for early but incomplete bone marrow engraftment.

Bone marrow recovery in the patients receiving autologous transplantation tended to lag behind that in patients receiving syngeneic transplantation. The mean days of granulocyte, platelet, and reticulocyte count recovery in the seven evaluable autologous transplant recipients (untreated and 4-hydroperoxycyclophosphamide-treated) were 21 (range, 11-59), 52 (range, 35-89), and 31 (range, 19-73), respectively, compared to 19 (range, 13-24), 24 (range, 20-47), and 24 (range, 17-28), respectively, in the eight evaluable syngeneic transplant recipients. This delay in recovery of the autologous transplant recipients may have been due in part to the effect of bone marrow incubation in vitro with 4-hydroperoxycyclophosphamide. In the five evaluable patients receiving autologous rescue with bone marrow incubated in vitro with 4-hydroperoxycyclophosphamide, the mean days of granulocyte, platelet, and reticulocyte count recovery were 24 (range, 16-59), 63 (range, 35-89), and 33 (range, 20-73), respectively, compared to 19 (range, 11-31), 25 (range, 20-52), and 24 (range, 17-31), respectively, in the ten evaluable patients who received syngeneic or autologous bone marrow rescue without the use of 4-hydroperoxycyclophosphamide.

Infectious Complications

All of the patients became febrile during the period of their granulocytopenia, and were treated with broad-spectrum antibiotics. Only five of the 19 patients developed documented bacteremias, one with *Pseudomonas aeruginosa,* one with *Klebsiella oxytoca,* one with *Streptococcus viridans,* and two with *Staphylococcus epidermidis*; all patients responded to appropriate antibiotic therapy. Four patients developed severe viral infections; one patient had a cytomegalovirus hepatitis, while three of the four patients with lymphoma developed a fatal diffuse interstitial pneumonitis, two with herpes simplex and one with disseminated herpes zoster. Herpes simplex stomatistis was seen in seven patients.

Nonhematologic Toxic Effects

Drug-related toxic effects during the first 30 days following bone marrow infusion were evaluated by chart review, using standard Eastern Cooperative Oncology Group (ECOG) criteria (26) (table 2). All 19 patients had mild to moderate nausea and vomiting with the use of vigorous antiemetic therapy. Sixteen of the 19 patients had mild to moderate diarrhea. Stomatitis was mild in three patients and severe in 14 (so that they were unable to eat), of whom seven had documented herpes simplex stomatitis. Mild to moderate desquamation of the skin was noted in most patients in the intertriginous areas (ie, axillary, inguinal).

Nine of the patients had mild to moderate renal impairment, with a median peak creatinine level of 1.6 mg/100 ml (range, 1.3-2.9); by Day 30, all of the abnormal levels were improving or normal, except for two patients who developed hepatorenal syndrome on the basis of veno-occlusive disease (VOD). Only two patients had clinically significant hemorrhagic cystitis. No clinically significant cardiac toxicity was seen; although serial ECGs were not uniformly obtained, five of the six evaluable patients had sinus arrhythmia or nonspecific ST-T wave changes. None of the six instances of pulmonary complications was directly attributable to drug-related toxicity; one patient had a probable bacterial pneumonia (pulmonary infiltrate with concomitant bacteremia), three had diffuse interstitial pneumonitis with disseminated herpes virus infection, one had fluid overload, and one had an infiltrate attributed to aspiration of blood. In terms of CNS complications, one patient had a generalized seizure following a dose of cyclophosphamide (with a normal serum sodium level), one had a transient episode of obtundation (perhaps related to transient hepatic failure), and one had an encephalitis attributed to disseminated herpes zoster infection.

Hepatic function abnormalities (table 3) following bone marrow infusion are presented for those patients with normal values at the time of treatment with busulfan. Analysis was limited to the first 30 days following bone marrow transplantation to avoid possible con-

TABLE 2.—Nonhematologic toxic effects during the 1st 30 days*

Toxic effect	ECOG grade of—					Total
	0	1+	2+	3+	4+	
Stomatitis	2	2	1	14	0	19
Renal (peak creatinine)	10	5	4	0	0	19
Cardiac (ECG)	1	5	0	0	0	6

*Values = No. of patients.

founding of data by viral hepatitis from transfusion therapy. Eight of 18 patients had elevations in alkaline phosphatase; by Day 30, all of these were resolving toward normal or were remaining stable. Six of 19 patients had elevations in bilirubin; by Day 30, three were normalizing and three were rising. Sixteen of 18 patients had elevations in SGOT; by Day 30, only four were not falling toward normal. Both patients with an increased level of alkaline phosphatase or bilirubin prior to initiation of busulfan had normalizing levels by Day 30.

Hepatic VOD was fatal in two patients (UPN 209 and 296), with pathologic findings at postmortem examination. Both patients had elevated and rising bilirubin at Day 30, but it should be noted that a third patient (UPN 212) had elevated and rising bilirubin, alkaline phosphatase, and SGOT with transient obtundation, but recovered completely. Otherwise, initial, peak, Day 30, and trend of hepatic function parameter levels at Day 30 were not predictive of fatal VOD.

Tumor Response

Nine patients were treated while in complete remission, and therefore had no measurable tumor (table 1). Of the ten patients with measurable tumor, all had an objective response to this regimen. Both of the patients with AML achieved a complete remission, although one was of short duration. The patients with ALL and AMF both achieved complete remission, also of short duration. Both patients with CML were rendered Philadelphia-chromosome-negative; the patient in blast crisis died of hepatic VOD, but the second patient remained Philadelphia-chromosome-negative for > 1 year. All four patients with lymphoma achieved a complete remission, although three died during the peritransplantation period of diffuse interstitial pneumonitis from disseminated herpes virus infection; the fourth patient relapsed after a short period of time.

In terms of duration of tumor control, 14 of the 19 patients were evaluable, five having died of the complications of the transplantation procedure. Eight patients eventually relapsed at a median of 114 days (range, 75–482) after transplantation. The other six patients (five of whom had no measurable tumor) remain in complete remission 158 to 1234 days after transplantation.

DISCUSSION

Although this group of patients was heterogeneous in terms of diagnosis, age, previous therapy, status of disease prior to treatment, and type of bone marrow rescue, all were evaluable for the toxicity of high-dose busulfan and cyclophosphamide, and tumor response without the confounding allogeneic transplantation effects of graft-versus-host and graft-versus-leukemia, respectively.

Severe myelosuppression with its attendant infectious complications was expected and manageable, with leukocyte and platelet count recovery occurring at a range of 11–59 and 20–89 days, respectively. All five patients who died relatively early in the posttransplant period, prior to full recovery of peripheral blood cell counts, had evidence for early bone marrow engraftment. The kinetics of hematologic reconstitution in this study is similar to that following allogeneic transplantation with the same regimen. Since allogeneic transplant patients uniformly recover with donor type cells, we feel that recovery in the autologous setting is from transplanted marrow (27).

Nausea, vomiting, and diarrhea were frequent but readily managed with vigorous medical therapy. Stomatitis was severe in 14 patients, but seven of them had a

TABLE 3.—Hepatic toxic effects during the 1st 30 days in patients with normal parameters on admission*

Parameter	Maximum value compared to normal				Total
	< 1.5×	1.5–2.0×	2.1–5.0×	> 5.0×	
Alkaline phosphatase	10	2	5	1	18
Bilirubin	13	0	5	1	19
SGOT	2	2	8	6	18

*Values = No. of patients.

documented herpes simplex infection. Skin, renal, cardiac, pulmonary, and central nervous system complications attributable solely to drug-related toxicity were uniformly transient and non-life-threatening. There was no evidence that toxicity differed in those patients receiving intrathecal methotrexate as opposed to intrathecal cytarabine.

Hepatic function abnormalities were common but tended to be transient, with the exception of the two patients with hepatic VOD who died of irreversible hepatic and renal failure. In allogeneic bone marrow transplant recipients prepared with high-dose busulfan and cyclophosphamide, VOD was noted in all five patients given 20 mg/kg of busulfan over 4 days and in two of four patients given 16 mg/kg over 4 days who came to autopsy, by Beschorner et al (28). However, VOD is not unique to high-dose busulfan and cyclophosphamide: it has also been noted with high-dose cytarabine, carmustine, or dimethylbusulfan in combination with cyclophosphamide plus total-body irradiation (28); high-dose cyclophosphamide alone or with total-body irradiation, total-body irradiation alone, and the combination of high-dose carmustine, cytarabine, cyclophosphamide, and 6-thioguanine (29); high-dose carmustine, cyclophosphamide, cytarabine and total-body irradiation (30); and high-dose mitomycin as a single agent (31). In our patients, the sporadic nature of occurrence of VOD may be related to the cumulative effect of previous intensive cytotoxic therapy, undetected viral hepatitis, individual differences in absorption and metabolism of the high doses of preparative agents, or other factors.

Acute diffuse interstitial pulmonary fibrosis was not seen in our patients. However, three patients with lymphoma died of a diffuse interstitial pneumonitis with concurrent disseminated herpes virus infection. Diffuse herpes virus pneumonitis is not unique to high-dose busulfan and cyclophosphamide, but has also been noted in other patients with lymphoma treated with intensive myelosuppressive chemotherapy. That this occurred only in patients with lymphoma may suggest that a defect in cell-mediated immunity inherent to the underlying disease process and intensive chemotherapy with its attendant intensive myelosuppression and immunosuppression are important in the development of the diffuse interstitial pneumonitis.

With daily oral low-dose busulfan, other alleged drug-related side effects have included gynecomastia (3), testicular atrophy (4), urticaria (4), erythema nodosum (4), hyperuricemia with nephrolithiasis (4), porphyria cutanea tarda (6), posterior subcapsular cataracts (7), myasthenia gravis (8), prolonged atrioventricular conduction time (9), erythema multiforme (10), cholestatic jaundice (11), portal hypertension (12), endocardial fibrosis (13), sicca syndrome (14), hemorrhagic cystitis (15), and widespread cytologic dysplasia (16). These reported side effects have not been observed in our group of patients, but our experience has been limited by the relatively brief duration of follow-up in many cases.

At intermediate doses, busulfan has been demonstrated to have an antineoplastic effect in several studies. Sykes (18) obtained an objective response (including two complete remissions) in six of 21 patients with lymphoma treated with 25 mg/day of busulfan for 2-4 days. Bean (19) induced complete remission in all four patients with chronic lymphocytic leukemia treated with a single oral 200-mg dose of busulfan. Sullivan (20) noted objective tumor regression in six of 17 patients with bronchogenic carcinoma treated with 0.5-2.0 mg/kg/day of busulfan for 2-4 days; pancytopenia was the predominant toxic effect. Arduino and Mellinger (21) noted an objective response in one patient with advanced carcinoma of the prostate who was treated with busulfan at an escalating schedule to a total dose of 348 mg over 33 days; pancytopenia was ultimately fatal.

In the present study, all of the patients treated in relapse or without previous therapy with high-dose busulfan and cyclophosphamide achieved a complete remission. It should be noted that experience with oral busulfan at low doses suggests that bioavailability may be erratic; this question is currently being addressed at our institution by measuring serum busulfan levels in patients receiving this regimen with a newly developed assay. Preliminary results suggest that plasma levels of busulfan vary less than fourfold. Nevertheless, these treatment results, coupled with the finding that most of our 19 patients tolerated this regimen with manageable side effects, suggest that high-dose busulfan and cyclophosphamide with autologous bone marrow rescue should be considered for phase II clinical trials in leukemia and carcinoma.

REFERENCES

1. GALTON DA. Myleran in chronic leukaemia. Results of treatment. Lancet 1:208, 1953.
2. HAUT A, ALTMAN SJ, CARTWRIGHT GE, ET AL. The use of Myleran in the treatment of chronic myelocytic leukemia. Arch Intern Med 96:451, 1955.
3. GALTON DA, TILL M, and WILTSHAW E. Busulfan (1,4-dimethanesulfonyloxybutane, Myleran): summary of clinical results. Ann NY Acad Sci 68:967, 1958.
4. KYLE RA, SCHWARTZ RS, OLINER HL, ET AL. A syndrome resembling adrenal cortical insufficiency associated with long term busulfan (Myleran) therapy. Blood 18:497, 1961.
5. OLINER H, SCHWARTZ R, RUBIO F, ET AL. Interstitial pulmonary fibrosis following busulfan therapy. Am J Med 31:134, 1961.
6. KYLE RA, and DAMESHEK W. Porphyria cutanea tarda associated with chronic myelogenous leukemia treated with busulfan. Blood 23:776, 1964.
7. SMALLEY RV, and WALL RL. Two cases of busulfan toxicity. Ann Intern Med 64:154, 1966.
8. DJALDETTI M, PINKHAS J, DEVRIES A, ET AL. Myasthenia gravis in a patient with chronic myeloid leukemia treated by busulfan. Blood 32:336, 1968.

9. HARROLD BP. Syndrome resembling Addison's disease following prolonged treatment with busulfan. Br Med J 1:463, 1966.
10. DOSIK H, HUREWITZ DJ, ROSNER F, ET AL. Bullous eruption and elevated leukocyte alkaline phosphatase in the course of busulfan-treated chronic granulocytic leukemia. Blood 35:543, 1970.
11. UNDERWOOD JC, SHAHANI RT, and BLACKBURN EK. Jaundice after treatment of leukaemia with busulfan. Br Med J 1:556, 1971.
12. FOADI MD, SHAW S, and PARADINAS FJ. Portal hypertension in a patient with chronic myeloid leukaemia. Postgrad Med J 53:267, 1977.
13. WEINBERGER A, PINKHAS J, SANDBANK U, ET AL. Endocardial fibrosis following busulfan treatment. JAMA 231:495, 1975.
14. SIDI Y, DOUER D, and PINKHAS J. Sicca syndrome in a patient with toxic reaction to busulfan. JAMA 238:1951, 1977.
15. MILLARD RJ. Busulfan haemorrhagic cystitis. Br J Urol 50:210, 1978.
16. WARD HN, KONIKOV N, and REINHARD EH. Cytologic dysplasia occurring after busulfan (Myleran) therapy. Ann Intern Med 63:654, 1965.
17. LIVINGSTON RB, and CARTER SK. Single Agents in Cancer Chemotherapy. New York, IFI/Plenum, 1970, pp 116-125.
18. SYKES MP. Myleran in the treatment of lymphomas. Ann NY Acad Sci 68:1035, 1958.
19. BEAN RHD. Myleran in the treatment of chronic lymphatic leukemia. Isr J Med Sci 1:801, 1965.
20. SULLIVAN RD. Myleran therapy in bronchogenic carcinoma. Ann NY Acad Sci 68:1038, 1958.
21. ARDUINO LJ, and MELLINGER GT. Clinical trial of busulfan (NSC-750) in advanced carcinoma of the prostate. Cancer Chemother Rep 51:295-303, 1967.
22. TUTSCHKA PJ, ELFENBEIN GJ, SENSENBRENNER LL, ET AL. Preparative regimens for marrow transplantation in acute leukemia and aplastic anemia. Am J Pediatr Hematol Oncol 2:363, 1980.
23. THOMAS ED, and STORB R. Techniques for human grafting. Blood 36:507, 1970.
24. APPELBAUM FR, HERZIG GP, ZIEGLER JL, ET AL. Successful engraftment of cryopreserved autologous bone marrow in patients with malignant lymphoma. Blood 52:85, 1978.
25. KAIZER H, STUART RK, FULLER DJ, ET AL. Autologous bone marrow transplantation in acute leukemia: progress report on a phase I study of 4-hydroperoxycyclophosphamide (4HC) incubation of marrow prior to cryopreservation. Proc ASCO 23:131, 1982.
26. OKEN MM, CREECH RH, TORMEY DC, ET AL. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649, 1982.
27. TUTSCHKA PJ, SANTOS GW, and ELFENBEIN GJ. Marrow transplantation in acute leukemia following busulfan and cyclophosphamide. Blut(suppl 25):375, 1980.
28. BESCHORNER WE, PINO J, BOITNOTT JK, ET AL. Pathology of the liver with bone marrow transplantation. Am J Pathol 99:369, 1980.
29. BERK PD, POPPER H, KRUEGER GR, ET AL. Veno-occlusive disease of the liver after allogeneic bone marrow transplantation. Ann Intern Med 90:158, 1979.
30. WOODS WG, DEHNER LP, NESBIT ME, ET AL. Fatal veno-occlusive disease of the liver following high dose chemotherapy, irradiation and bone marrow transplantation. Am J Med 63:285, 1980.
31. SARNA GP, CHAMPLIN R, WELLS J, ET AL. Phase I study of high-dose mitomycin with autologous bone marrow support. Cancer Treat Rep 66:277-282, 1982.

Evaluation of Aminoglutethimide in Stage D Prostate Cancer: An Assessment of Efficacy and Toxicity in Patients With Tumors Refractory to Hormonal Therapy[1]

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Prostatic carcinoma is the most common visceral malignancy in men > 50 years of age. Once metastatic disease appears, median survival is 1-3 years (1). Exogenous estrogen therapy or orchiectomy may produce dramatic clinical improvement and regression of metastatic lesions in 70%-80% of patients (2). However, prostatic adenocarcinoma which is progressing despite testicular androgen suppression is infrequently responsive to secondary hormonal therapies; the median survival of such patients is only 6 months (3). An array of hormonal and cytotoxic therapies have been tried in such patients and all have had limited success (4-6).

Several investigators have reported benefit from suppressing adrenal androgen secretion in patients with prostate cancer, although the role of adrenal androgens in supporting the growth of prostatic carcinoma is unclear. In normal men, the androgens synthesized by the adrenals are androstenedione (3 mg/day) and dehydroepiandrosterone (24 mg/day) (4). Although these compounds are only one-tenth as potent as testosterone, their levels may increase 100-fold after orchiectomy (7). Surgical adrenalectomy has been reported to induce clinical responses in 20%-40% of patients with stage D carcinoma of the prostate after relapse from conventional hormonal therapy (8). Preliminary reports suggest that medical adrenalectomy with aminoglutethimide (AG) may be associated with responses or disease stabilization in almost 50% of patients (9-12). This study describes the results of AG therapy in patients with advanced prostate cancer which was refractory to standard hormonal manipulations. AG and adrenal androgen plasma concentrations were determined prospectively to evaluate patient compliance and to determine completeness of adrenal suppression.

METHODS

Twenty-three patients with metastatic prostate cancer which was progressive and symptomatic after at least one hormonal treatment were entered in the study; 20 patients were evaluable. Three patients were inevaluable because they received AG for ⩽ 14 days and developed medical complications necessitating AG discontinuation. Informed consent was obtained from all patients. Eighteen of 20 evaluable patients had undergone orchiectomy and had progressive disease prior to initiation of AG therapy. Initial therapy for all patients was as follows: AG four times a day (250 mg orally), and cortisone acetate at 8 AM and 4 PM (10 mg) and at 10 PM (20 mg). This schedule of corticoid replacement was chosen to attempt to attain maximal suppression of ACTH secretion and to correspond to schedules reported by other investigators. Mineralocorticoid was not routinely prescribed. Plasma dehydroepiandrosterone sulfate (DHEAS) levels were determined prior to initiation of therapy, 2 and 4 weeks later, and then monthly. AG doses were adjusted in an attempt to achieve maximal DHEAS suppression. AG concentrations were determined 2 hours after the morning dose of AG. If DHEAS levels were not suppressed to ⩽ 5% of the pretreatment level and the AG level was ⩽ 15 μg/ml, the AG dose was increased by 250-mg increments every 2 weeks. Electrolytes, cbc, SMA-12, and acid phosphatase levels were measured regularly.

AG was assayed by a modification of the Bratton and Marshall procedure (13) for diazotizable aromatic amines, which was adapted for the Technicon Autoanalyzer (14). Serum (0.7-1.0 ml) was sequentially extracted with chloroform and 0.5 M hydrochloric acid. The HCl layer was transferred to Autoanalyzer cups and assayed as previously described.

Commercially available antisera for determination of DHEAS and ^{3}H-DHEA were employed (Radioassay Systems Labs and New England Nuclear Corp). Radioimmunoassay procedures followed the methods of Manlimos and Abraham (15). Response was assessed utilizing the criteria of the National Prostatic Cancer Project (16).

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RESULTS

Clinical Characteristics

Clinical characteristics of these patients are summarized in table 1. The median age was 64 years. All patients had received at least one hormonal therapy for prostate cancer and their tumors had become refractory to this treatment. These patients were heavily pretreated: only four patients had received only one prior systemic therapy for prostate cancer (orchiectomy). Seven patients had received two, six had received three, one had received four, and one had received seven different previous systemic therapies. Seven patients had previously received systemic cytotoxic chemotherapy. Twelve patients were ambulatory, seven were confined to bed < 50% of the time, and one was confined to bed > 50% of his waking hours. Nineteen patients had prominent bone metastases; in addition, 12 patients had lymph node or soft tissue metastases, including one with pleural effusion, two with chest wall metastases, two with extrapleural metastases, and one with breast metastases. Acid phosphatase was increased in 19 of 23 patients and in 17 of 20 evaluable patients. Primary prostatic tumors had originally been described as poorly differentiated in eight patients, moderately well-differentiated in eight, and well-differentiated in one. Characterization of the grade of the primary tumor was not available in three patients.

TABLE 1.—Clinical characteristics of patients evaluable for response

Characteristic	No. of patients
Total evaluable	20
Median age in yrs: 64	
Previous orchiectomy	18
Previous hormonal therapy	20
Performance status (ECOG)*	
0	0
1	12
2	7
3	1
4	0
Major sites of disease	
Bone	19
Visceral	2
Lymph node	9
Soft tissue	6
Local recurrence	7
Histologic differentiation	
Poor	8
Moderate	8
Well-differentiated	1
Unknown	3
No. of previous therapies (excluding radiotherapy)	
0	0
1	4
2	7
3	7
4	1
> 4	1

* ECOG = Eastern Cooperative Oncology Group.

Clinical Response

Twenty-three patients entered this trial and began AG and cortisone acetate as described. Three patients were treated for 5, 8, and 14 days and were declared inevaluable. Two of these patients developed severe pneumonia shortly after beginning therapy. In two patients, precipitous decreases in platelet count were noted within 48–72 hours of beginning AG. The platelet count fell as low as 36,000 and 34,000/mm^3, respectively, in these two patients. Thrombocytopenia persisted despite cessation of AG. Both patients had extensive marrow infiltration by tumor and one had severe pneumonia.

The 20 evaluable patients received AG for a median of 52 days (range, 14–164). In these patients, AG was discontinued because of progressive disease. Seventeen of 20 evaluable patients received AG for ≥ 30 days; the remaining three patients received AG for 14, 15, and 16 days, respectively. AG was discontinued because of obvious progressive disease.

Eight patients received escalated doses of AG. Our target of suppression of DHEAS to < 5% of pretreatment level was achieved in ten of 17 patients in whom an adequate number of determinations were performed. Fourteen patients had DHEAS suppressed to ≤ 50% of basal levels. Twenty evaluable patients received ≥ 1 g of AG/day for ≥ 14 days; no responses were seen. No patient appeared to benefit from therapy with AG. All patients with measurable disease had clear-cut progression of their metastases and no patient with evaluable disease only was felt to have improved based on either subjective criteria or ancillary laboratory data. No decrease in serum acid phosphatase was noted during therapy.

AG Pharmacology

After receiving 1 g of AG/day for 14 days, mean AG plasma concentration in ten patients was 6.33 μg/dl. After 4 weeks, mean AG concentration was 6.92 μg/dl in seven patients receiving 1 g; at 6 weeks, mean AG concentration was 5.59 μg/dl in five patients receiving 1 g of AG. These data do not suggest that metabolism of AG changed substantially between 2 and 6 weeks on therapy. While the data are limited, plasma AG concentration after 2 weeks in patients receiving 1 g/day was not apparently related to body surface area (m^2) or weight (kg) ($r = -0.2$).

Plasma AG data are limited in patients receiving > 1 g/day. Doses of AG were increased in eight patients. Six of eight patients receiving > 1 g/day of AG achieved ≥ 95% suppression of DHEAS; however, DHEAS was suppressed to undetectable levels in only three patients.

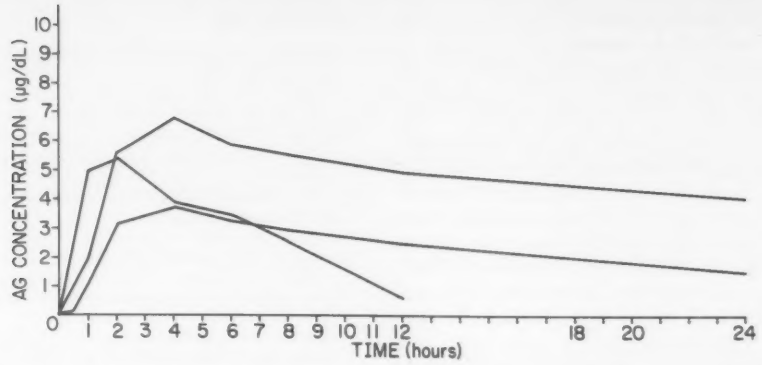

FIGURE 1.—Plasma concentrations of AG, following oral ingestion of 500 mg; data were obtained prior to initiation of therapeutic schedule of AG.

Despite doses up to 1750 mg/day, AG concentrations > 9.8 µg/dl were not achieved in any patients.

Prior to therapeutic administration of AG, plasma concentrations of AG were measured in three patients, following the ingestion of 500 mg of AG. Acute response to AG was variable: peak plasma AG levels occurred between 2 and 6 hours after AG ingestion (fig 1). Peak AG concentrations were 3.7, 5.4, and 6.8 µg/dl. Plasma half-lives of AG were also quite variable in these three patients. Half-lives of 6, 15½, and > 20 hours were noted. It is of interest that the highest AG plasma concentration noted in this study occurred in the patient with a half-life > 20 hours. This patient had a plasma AG concentration of 14 µg/dl after 2 weeks of 1 g/day of AG. Marked lethargy, hypotension, and hyponatremia and hyperkalemia were evident at that time. These abnormalities responded to mineralocorticoid replacement, increased glucocorticoid dose, and reduced doses of AG.

Toxic Effects

The toxic effects of AG were modest, as shown in table 2. Fourteen evaluable patients (73%) and all three inevaluable patients experienced some toxic effects. Mild (ECOG grade ⩽ 2) asymptomatic hepatic enzyme elevation (SGOT) occurred in ten patients. No patient required cessation or alteration of the therapy for biochemical evidence of hepatic dysfunction. Electrolyte disturbances indicative of mineralocorticoid deficiency occurred in three patients, and were managed with the administration of Fluoronef (0.1 mg/day). Skin rash occurred in three patients. Three patients experienced lassitude, one developed a mild cushingoid habitus, and one noted mild hirsutism. No definite hematologic toxicity was seen. Two patients had treatment discontinued secondary to marked thrombocytopenia, which was felt to be secondary to prostate cancer.

DISCUSSION

AG, a derivative of the hypnotic glutethimide, was introduced in 1959 as an anticonvulsant. Use of this drug virtually ceased a few years later, when it was found to cause goitrous hypothyroidism and adrenal insufficiency. Recently, however, the ability of AG to suppress adrenal function has led to increased interest in its use. AG inhibits the desmolase reaction in the adrenal, thereby preventing the conversion of cholesterol to delta 5-pregnenolone (17). Histologic examination of the adrenal glands of rats and humans after treatment with AG reveals heavy, pale glands which show cellular hypertrophy, cytoplasmic vacuolation, and excessive accumulation of lipid (17,18). AG also inhibits the aromatase reaction, thus blocking the peripheral conversion of androstenedione to estrone (19). Direct inhibition of testicular steroidogenesis has also been reported (20). AG has also been shown to block thyroxine synthesis (21).

Studies in rats with Dunning prostatic cancer suggest a possible role for adrenal ablation in the treatment of prostatic carcinoma (22). Results of clinical trials, however, have been variable; surgical adrenalectomy has not been widely practiced (8,23). The availability of drugs similar to AG makes a "medical adrenalectomy" possible. Such a procedure is appealing because of its relative ease

TABLE 2.—Toxic effects of AG and cortisone acetate in 23 patients

Toxic effect	No. of patients (%)
Total with toxic effects	17 (85)
Hepatic enzyme elevation (SGOT)	11 (55)
Electrolyte disturbances	3 (15)
Skin rash	3 (15)
Lassitude	3 (15)
Hirsutism	1 (5)
Cushingoid habitus	1 (5)
Thrombocytopenia	2 (9)*

* Not conclusively attributable to AG.

and reportedly manageable toxicity. Preliminary reports have suggested benefit from medical adrenalectomy in patients with prostatic carcinoma. Robinson et al (11) treated 26 patients with AG (500–1000 mg/day) and diethylstilbestrol (3 mg/day). Twelve of 20 patients who had failed to respond to estrogen therapy were subjectively improved. Objective responses were seen in six patients; however, five of these were given AG and diethylstilbestrol as initial therapy for prostate cancer. Seventy-three percent of their patients experienced toxic effects, and in eight (31%), treatment had to be terminated. Sanford and co-workers (10) initially reported subjective improvement following AG treatment in three of seven patients who had failed to respond to orchiectomy and exogenous estrogen therapy. This group has most recently reported results in 44 patients; objective responses in 15% of the patients were described (one complete response; five partial responses) (9).

Contrary to these reports, we have found no benefit of AG in our patients with prostatic carcinoma who had failed standard hormonal therapy. A criticism of our study is that our patients were very heavily pretreated. All patients had failed primary hormonal manipulation and 16 of 20 evaluable patients had received other systemic therapy following primary hormonal manipulation. This must certainly influence the lack of clinically important activity noted in our trial. Lippman and co-workers (24) have also reported lack of efficacy of AG in 12 patients with advanced prostate cancer.

It is not clear what level of adrenal suppression might be necessary for therapeutic benefit in prostate cancer. In these heavily pretreated patients, moderate adrenal suppression (30%–50%) was uniformly obtained and in ten of 17 patients, DHEAS concentrations were reduced by $\geq$ 95%. Nonetheless, no therapeutic benefit was noted.

It is noteworthy that the pharmacokinetics of AG seem to be variable in men with prostate cancer. Plasma half-lives varied more than threefold in the three patients in whom it was determined. Mean plasma levels in our group of men were also substantially lower than those previously noted in women with breast cancer. Murray et al (25) found a mean drug level of 12.04 μg/dl at 2 weeks in postmenopausal or castrated women, compared to a concentration of 6.3 μg/dl in our men receiving an identical dose. The reason for this discrepancy is not immediately apparent, but may be related to the difference in sex between the two study populations.

From our study, we conclude that any future studies of AG in men with prostate cancer should be restricted to earlier stages of the disease and should be aware of the interpatient variations in AG metabolism noted. Administered at "standard doses," AG appears to offer little benefit for men with prostate cancer which has progressed following primary hormonal manipulation and secondary therapeutic attempts.

REFERENCES

1. WHITMORE WF. The natural history of prostate carcinoma. Cancer 32:1104–1112, 1973.
2. RESNICK MT, and GRAYHACK JJ. Treatment of stage IV carcinoma of the prostate. Urol Clin North Am 2:141–161, 1978.
3. KLEIN LA. Prostatic carcinoma. N Engl J Med 300:824–833, 1979.
4. SCOTT WW, MENON M, and WALSH PC. Hormonal therapy of prostatic cancer. Cancer 45:1929–1936, 1980.
5. YAGODA A. Non-hormonal cytotoxic agents in the treatment of prostatic carcinoma. Cancer 32:1131–1140, 1973.
6. SCHMIDT JD. Chemotherapy of hormone-resistant stage D prostatic cancer. J Urol 123:797–805, 1980.
7. BEACH PD. Hormonal therapy for prostatic carcinoma. *In* Cancer of the Genitourinary Tract (Johnson DE, and Samuels ML, eds). New York, Raven Press, 1979, vol 23, pp 273–278.
8. BHANALAPH T, VARKARAKIS MH, and MURPHY GP. Current status of bilateral adrenalectomy for advanced prostatic carcinoma. Ann Surg 179:17–23, 1974.
9. WORGUL TJ, SANTEN RJ, SAMOJLIK E, ET AL. Clinical and biochemical effect of aminoglutethimide in the treatment of advanced prostatic carcinoma. Proc Am Assoc Cancer Res and ASCO 22:471, 1981.
10. SANFORD EJ, DRAGO JR, ROHNER TJ, ET AL. Aminoglutethimide medical adrenalectomy for advanced prostatic carcinoma. J Urol 115:170–174, 1976.
11. ROBINSON MRG, SHEARER RJ, and FERGUSSON JD. Adrenal suppression in the treatment of carcinoma of the prostate. Br J Urol 46:555–559, 1974.
12. ROBINSON MRG. Aminoglutethimide: medical adrenalectomy in the management of carcinoma of the prostate. A review after six years. Br J Urol 52:328–329, 1980.
13. BRATTON AC, and MARSHALL EK, JR. New coupling component for sulfanilamide determination. J Biol Chem 128:537, 1939.
14. AREND RA, LEKLEM JE, and BROWN RR. Direct and steam distillation autoanalyzer methods for assay of diazotizable aromatic amine metabolites of tryptophan in urine and serum. Biochem Med 4:457, 1970.
15. MANLIMOS FS, and ABRAHAM GE. Anal Lett 8:403, 1975.
16. SCHMIDT JD, JOHNSON DE, SCOTT WW, ET AL. Chemotherapy of advanced prostatic cancer—evaluation of response parameters. Urology 7:602–610, 1976.
17. CASH R, BROUGH AJ, COHEN MNP, ET AL. Aminoglutethimide as an inhibitor of adrenal steroidogenesis: mechanism of action and therapeutic trial. J Clin Endocrinol 27:1239–1248, 1967.
18. MORALES A, CONNOLLY JG, MOBBS BG, ET AL. Effects of aminoglutethimide on the prostate glands of rats—a preliminary study. Can J Surg 14:154–160, 1971.
19. SAMOJLIK E, SANTEN RJ, and WELLS SA. Adrenal suppression with aminoglutethimide. II. Differential effects of aminoglutethimide on plasma androstenedione and estrogen levels. J Clin Endocrinol Metab 45:480–487, 1977.
20. SANTEN RJ, COHN N, MISBIN R, ET AL. Acute effects of aminoglutethimide on testicular steroidogenesis in normal men. J Clin Endocrinol Metab 49:631–634, 1979.
21. SANTEN RJ, WELLS SA, COHN N, ET AL. Compensatory increase in TSH secretion without effect in prolactin secretion in patients treated with aminoglutethimide. J Clin Endocrinol Metab 45:739–746, 1977.
22. SHESSEL FS, BLOCK NL, STOVER B, ET AL. Endocrine manipulation of the Dunning prostatic adenocarcinoma. Invest Urol 17:529–533, 1980.
23. MURPHY GP, SCHOONEES R, GAILANI S, ET AL. Hypophysectomy and adrenalectomy for disseminated prostatic carcinoma. J Urol 105:817–825, 1971.
24. LIPPMAN A, COHEN F, SAMOJLIK E, ET AL. Aminoglutethimide for metastatic prostate cancer. Proc ASCO 2:144, 1983.
25. MURRAY FT, SANTNER S, SAMOJLIK E, ET AL. Serum aminoglutethimide levels: studies of serum half-life, clearance and patient compliance. J Clin Pharmacol 19:704–711, 1979.

Human Leukocyte (Alpha) Interferon in Metastatic Malignant Melanoma: The American Cancer Society Phase II Trial[1,2]

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Forty-four evaluable patients with metastatic malignant melanoma confined to the skin, subcutaneous tissues, lymph nodes, and/or lung were randomly assigned to receive either 1×10^6, 3×10^6, or 9×10^6 units of partially purified human leukocyte (alpha) interferon by daily im injection for 42 days. One patient achieved a partial response, two had minor responses, and three others had mixed responses. The only partial response was observed at the lowest dose of interferon. Toxicity increased in frequency and intensity with increasing interferon dose. This preparation of interferon at the doses, route, and schedule used appears to have little efficacy in metastatic malignant melanoma. [Cancer Treat Rep 68:723–726, 1984]

Interferons are a family of inducible cellular proteins capable of inhibiting virus replication and cellular proliferation and of modifying a variety of immunologic responses that may be involved in regulating host-tumor interactions (1). They inhibit tumor growth and prolong survival in animal tumors (2,3), and recent clinical trials have also demonstrated activity against some malignant tumors in man (4–6). In human tumor stem cell assays, interferons have been shown capable of inhibiting the growth of tumor cell colonies from a variety of tumors including malignant melanoma (7).

Based on these observations, the American Cancer Society initiated a multi-institutional trial to evaluate the efficacy of human leukocyte (alpha) interferon in metastatic malignant melanoma, a tumor in which existing chemotherapeutic agents induce a response at low and inconsistent rates (8). Since the optimal dose of interferon was unknown, the trial was designed to include random assignment to one of three dose levels of interferon and to correlate therapeutic response and toxicity with the interferon dose.

MATERIALS AND METHODS

Human leukocyte interferon, prepared from buffy coat leukocytes, was obtained from Dr. Kari Cantell and the Finnish National Red Cross (9). It was partially purified to a specific activity of 10^6 units/mg of protein and was tested for potency and safety as described previously (4). Patients were stratified according to the presence or absence of lung metastases and were randomly assigned to receive either 1×10^6, 3×10^6, or 9×10^6 units of interferon by daily im injection for 42 days. Because of the limited supply of interferon and the observation that patients with lymphomas showed continued disease regression after treatment with interferon was discontinued (5), no patient was treated for > 42 days, regardless of response. Treatment was discontinued earlier if there was objective evidence of tumor progression. Dose reduction (to one-third of the original dose) was permitted for persistent neutropenia, persistent high fever, severe malaise, or anorexia.

Patients were required to have biopsy-proven, bidimensionally measurable, recurrent or metastatic malignant melanoma with disease confined to the skin, subcutaneous tissues, lymph nodes, and/or lung. They were required to have a Karnofsky performance status (PS) of ≥ 50, life expectancy of at least 8 weeks, neutrophil count $\geq 1500/mm^3$, platelet count $\geq 100{,}000/mm^3$, and

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no cytotoxic chemotherapy, radiation therapy, immunotherapy, or hormonal therapy (including corticosteroids) within 4 weeks of study entry.

Forty-five patients were entered in the study: 17 from the Memorial Sloan-Kettering Cancer Center, 16 from the University of California at Los Angeles, and 12 from the Yale University School of Medicine. One patient who was found to have brain metastases 4 days after starting interferon and was then treated with corticosteroids and radiation therapy was excluded from the analysis. Of the 44 evaluable patients, 22 had lung metastases and 22 had disease confined to the skin, subcutaneous tissues, or lymph nodes. There were 33 males and 11 females, with a median age of 51 years (range, 15–73). Thirty-six patients had no or minimal symptoms from their disease (PS, 90–100), and no patient had a PS < 70. Nineteen patients had received prior cytotoxic chemotherapy.

Patients were evaluated prior to treatment with complete history, physical examination, chest x-ray, measurement of marker lesion, cbc, wbc count with differential, prothrombin time, 10-channel biochemical screen, 5′-nucleotidase, gamma glutamyl transpeptidase, blood glucose, urinalysis, and additional scans and x-rays as required to define the extent of disease. During treatment, blood cell counts were repeated at least twice weekly, biochemical tests and measurements of palpable tumors were repeated at least weekly, and chest x-rays were repeated every 2–4 weeks. Skin test reactivity to 2,4-dinitrochlorobenzene and microbial antigens (dermatophytin-O, mumps, purified protein derivative, and streptokinase/streptodornase) was determined before and after treatment as described previously (10,11). Serum interferon activity was assayed before and during treatment using either vesicular stomatitis virus plaque reduction in human skin fibroblasts (12) or a cytopathic effect inhibition assay (13) and was compared to a National Institutes of Health alpha interferon standard. Partial response was defined as a > 50% decrease in the sum of the products of the longest perpendicular diameters of all measurable lesions lasting at least 1 month, without the appearance of new lesions; minor response was defined as a 25%–50% decrease in all measurable lesions; stable disease was defined as a < 25% change during the course of treatment, without the appearance of new lesions; and disease progression was defined as a > 25% increase or the appearance of new lesions.

RESULTS

Sixteen patients were treated at a dose of 1×10^6 units/day, and 14 each were treated at doses of 3×10^6 and 9×10^6 units/day. One patient, with extensive cutaneous metastases in the neck, showed objective partial response lasting 5 weeks at a dose of 1×10^6 units/day. She had previously had a variety of local treatments, including 5-FU cream, intralesional injections of BCG and 2,4-dinitrochlorobenzene, radiation therapy, and hyperthermia, but she had received no prior systemic treatment. Two previously untreated patients with lung metastases showed minor responses. One of these, treated at a dose of 1×10^6 units/day, showed complete regression of one of two lung metastases and five of six lymph node metastases and remained stable without further treatment for > 9 months, but eventually showed disease progression. The other patient, treated at a dose of 9×10^6 units/day, showed approximately a 40% decrease in the size of lung lesions followed by disease progression 1 month after treatment was discontinued.

Three other patients with disease confined to the skin, subcutaneous tissues, or lymph nodes showed mixed evidence of disease regression which did not qualify as a partial or minor response. All three patients had regional disease recurrence and had received prior therapy; their respective treatments were: regional perfusion with melphalan; BCG vaccination and perfusion with mechlorethamine; and local irradiation followed by systemic therapy with dacarbazine, bleomycin, vincristine, lomustine, and tamoxifen. The first of these patients, treated at a dose of 9×10^6 units/day, showed complete regression of 20 of 23 intradermal metastases but developed two new subcutaneous nodules at the same time. The second patient, treated at a dose of 3×10^6 units/day, developed pain and inflammation in all eight subcutaneous metastases, with a subsequent decrease of > 50% in the size of two of the lesions, while the other lesions showed no change. Biopsy of one of the regressing lesions showed only a few residual necrotic melanoma cells. The third patient, who was treated with 1×10^6 units/day, had widespread cutaneous disease on the right side of the neck and enlarged regional lymph nodes. During treatment, he developed an intense inflammatory reaction surrounding the skin lesions, with subsequent development of superficial ulcerations in and around the lesions and a minor decrease in the size of an enlarged supraclavicular lymph node. No further change occurred in the succeeding 3 months. He was lost to follow-up for 6 months, during which time he received one dose of melanoma membrane extract vaccine, several unknown compounds (reportedly containing selenium and sulfur), and a variety of vitamins. Examination after that period revealed healing of the ulcerations, flattening of the nodular pigmented lesions, and hypopigmentation of the surrounding skin. Multiple biopsies of the pigmented lesions showed infiltration of pigment-laden histiocytes, without residual melanoma cells. However, excision of a small left supraclavicular node revealed metastatic melanoma. Although we cannot attribute this patient's response with certainty to interferon, it is clear that the initial inflammatory response in the tumor developed during interferon treatment and before any other treatment was started.

Ten patients had stable disease during the course of treatment, and the remaining patients had disease progression.

Side effects were similar to those reported previously for partially purified human leukocyte interferon (4-6) and consisted primarily of fever, chills, malaise, anorexia and weight loss, and leukopenia. Occasionally, patients developed nausea and vomiting (usually on the first day of treatment in association with fever and chills) and, infrequently, diarrhea and headache. Many patients showed a decrease in blood pressure. This was usually asymptomatic, but in a few patients symptomatic orthostatic hypotension was observed. Several patients developed crusted labial lesions typical of herpes simplex infection within a few days of starting treatment. In all cases the patients had previously developed similar lesions during febrile illnesses. A moderate and reversible decrease in platelet counts and an increase in liver enzymes were observed frequently but were not dose-limiting.

Table 1 shows the relationship of the interferon dose to the observed side effects of 38 patients for whom full documentation is available. The frequency of moderate to severe fever and chills, malaise, nausea and vomiting, and hypotension increased with increasing interferon dose, as did the magnitude of wbc and neutrophil count depression and the increase in SGOT. Other side effects were not clearly related to the interferon dose. Four patients required dose reduction because of toxic effects. Two patients required dose reduction from 9×10^6 to 3×10^6 units/day because of leukopenia, and one required successive dose reductions from 9×10^6 to 3×10^6 and 1×10^6 units/day because of symptomatic orthostatic hypotension and nausea. The fourth patient had the dose reduced from 1×10^6 to 0.3×10^6 units/day because of severe malaise and progressive respiratory distress, which was probably attributable largely to disease progression. Seven patients failed to complete the 42-day course of treatment because of documented disease progression.

Of 44 patients tested for cutaneous delayed-type hypersensitivity reactions prior to interferon treatment, 39 (89%) showed a positive reaction to at least one antigen. Following treatment, 40 patients were re-tested and 34 (85%) showed at least one positive reaction. Of 140 paired individual skin tests performed before and after treatment, 107 (76%) showed no change, 15 (11%) changed from positive to negative, and 18 (13%) converted from negative to positive.

No patient had serum interferon activity detectable prior to treatment. Serum interferon activity was rarely detectable after interferon doses of 1×10^6 or 3×10^6 units. In patients treated at a dose of 9×10^6 units, variable levels of serum interferon activity were detected, with maximum titers on the first day of treatment ranging from 60 to 300 units/ml. Peak titers were generally observed from 4 to 12 hours after the first dose, and most patients treated at a dose of 9×10^6 units still had serum interferon activity detectable at 24 hours.

DISCUSSION

Using a partially purified preparation of human leukocyte (alpha) interferon, we observed only one partial and two minor responses in 44 evaluable patients with metastatic malignant melanoma. Three other patients showed mixed responses, with intense inflammation in the tumors in two; the third patient had complete regression of 20 of 23 skin nodules at the same time that new subcutaneous metastases developed. With such a low major objective response rate it is not possible to define the "op-

TABLE 1.—Side effects and interferon dose

	Interferon dose (units/day)		
	1×10^6	3×10^6	9×10^6
% of patients with moderate to severe—			
Fever, chills	36	54	85
Malaise	36	54	85
Nausea and vomiting	14	15	46
Hypotension (> 20 mm systolic)	36	54	67
Weight loss (> 10 lbs)	23	17	31
Herpetic lesions	7	0	8
Diarrhea	0	8	8
Headache	7	8	8
Mean nadir ± SE—			
Wbc count ($\times 10^{-3}/mm^3$)	4.36 ± 0.59	3.23 ± 0.28	2.34 ± 0.30
Absolute neutrophil count	2266 ± 312	1697 ± 213	976 ± 174
Absolute lymphocyte count	780 ± 129	629 ± 68	685 ± 102
Platelet count ($\times 10^{-3}/mm^3$)	188 ± 19	194 ± 19	140 ± 9
Mean maximum change ± SE in—			
SGOT	14.9 ± 4.1	36.8 ± 11.5	43.5 ± 12.3
Alkaline phosphatase	30.0 ± 8.8	33.3 ± 18.1	23.6 ± 6.4
5′-Nucleotidase	2.9 ± 0.9	13.3 ± 5.9	11.1 ± 2.8
Gamma glutamyl transpeptidase	48.7 ± 27.6	59.0 ± 32.6	47.4 ± 12.2

timal" dose of interferon, but it is of interest to note that the only partial response and one of the minor responses were observed in patients treated at the lowest (and least toxic) dose administered. Since interferon's effects on tumor growth may be mediated indirectly, there is no reason to expect, a priori, that higher doses would prove more effective. Golub et al (14), examining interferon-induced changes in natural killer (NK) cell cytotoxicity in a subset of the patients described in this paper, found that patients treated with the lowest dose of interferon showed sustained increases in NK activity throughout the course of treatment, with neither the acute decline in NK activity on Day 1 nor the gradual decline later in the course of treatment (after a peak increase at Day 7) observed in patients treated at doses of 3×10^6 or 9×10^6 units/day.

Considering the low objective response rate observed in this study, we cannot recommend the use of partially purified human leukocyte interferon, given at these doses on a daily schedule, as single-agent treatment for metastatic malignant melanoma. It must be recognized, however, that these patients were treated with an interferon preparation of low specific activity under conditions where the optimal dose, route, schedule, duration of treatment, and mechanism of action are unknown. Tumor regression has been noted in patients with cutaneous melanoma metastases whose tumors were directly injected with human leukocyte interferon at the same time that cimetidine (an H_2 receptor antagonist) was administered orally (15). In addition, major responses have been observed in patients with melanoma treated with higher doses of recombinant DNA-produced alpha interferons (16,17). Such findings suggest that interferon may prove to be effective in malignant melanoma once the basis of its action in the cancer patient is better understood and the optimal conditions for its use are defined.

REFERENCES

1. BORDEN EC, and BALL LA. Interferons: biochemical, cell growth inhibitory, and immunological effects. Prog Hematol 12:299-339, 1981.
2. GRESSER I, and TOVEY MG. Antitumor effects of interferon. Biochim Biophys Acta 516:231-247, 1978.
3. KRIM M. Towards tumor therapy with interferons, part II: interferons: in vivo effects. Blood 55:875-884, 1980.
4. BORDEN EC, HOLLAND JF, DAO TL, ET AL. Leukocyte-derived interferon (alpha) in human breast carcinoma. The American Cancer Society phase II trial. Ann Intern Med 97:1-6, 1982.
5. GUTTERMAN JU, BLUMENSCHEIN GR, ALEXANIAN R, ET AL. Leukocyte interferon-induced tumor regression in human metastatic breast cancer, multiple myeloma, and malignant lymphoma. Ann Intern Med 93:399-406, 1980.
6. MERIGAN TC, SIKORA K, BREEDEN JH, ET AL. Preliminary observations on the effects of human leukocyte interferon in non-Hodgkin's lymphoma. N Engl J Med 299:1449-1453, 1978.
7. SALMON SE, YOUNG L, and STEBBING N. Antitumor activity of natural and cloned human interferons (IF) in the human tumor stem cell assay (HTSCA). Proc Am Assoc Cancer Res and ASCO 22:290, 1981.
8. COSTANZA ME, NATHANSON L, SCHOENFELD D, ET AL. Results with methyl-CCNU and DTIC in metastatic melanoma. Cancer 40:1010-1015, 1977.
9. CANTELL K, HIRVONEN S, MORENSEN KE, ET AL. Human leukocyte interferon. *In* Production, Purification, Stability and Animal Experiments in the Production and Use of Interferon for the Treatment and Prevention of Human Virus Infections (Waymouth C, ed). Proceedings of a Tissue Culture Association Workshop. Rockville, MD, Tissue Culture Association, 1974, pp 35-38.
10. EILBER FR, and MORTON DL. Impaired immunologic reactivity and recurrence following cancer surgery. Cancer 25:362, 1970.
11. PINSKY CM. Skin tests. *In* Immunodiagnosis of Cancer (Herberman RB, and McIntire KR, eds). New York, Marcel Dekker, 1979, pp 722-738.
12. MERIGAN TC. A plaque inhibition assay for human interferon employing human neonate skin fibroblast monolayers and bovine vesicular stomatitis virus. *In* In Vitro Methods in Cell-Mediated Immunity (Bloom BR, and Glade PR, eds). New York, Academic Press, 1971, pp 489-499.
13. STEWART WE, II. Interferon assays. *In* The Interferon System. New York, Springer-Verlag, 1979, pp 17-19.
14. GOLUB SH, DOREY F, HARA D, ET AL. Systemic administration of human leukocyte interferon to melanoma patients. I. Effects on natural killer function and cell populations. JNCI 68:703-710, 1982.
15. BORGSTRÖM S, VON EYBEN FE, FLODGREN P, ET AL. Human leukocyte interferon and cimetidine for metastatic melanoma. N Engl J Med 307:1080-1081, 1982.
16. ERNSTOFF MS, REISS M, DAVIS CA, ET AL. Intravenous (iv) recombinant alpha-2 interferon (IFNα-2) in metastatic melanoma. Proc ASCO 2:57, 1983.
17. CREAGAN ET, AHMANN DL, GREEN SJ, ET AL. Recombinant leukocyte A interferon (RO 22-8181; rIFN-αA) in disseminated malignant melanoma (DMM). Proc ASCO 2:58, 1983.

Pharmacokinetics of Methotrexate in Adult Indian Patients and Its Relationship to Nutritional Status[1]

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Since anticancer drugs have a low therapeutic index, any significant change in the pharmacokinetics of a drug would have a bearing on therapeutic efficacy and drug toxicity. Nutritional deficiencies have been shown to affect the four processes of pharmacokinetics, ie, absorption, distribution, biotransformation, and excretion. Malnutrition and undernutrition are widely prevalent in India and thus it was thought to be of practical importance to study the effect of initial nutritional status on the overall kinetics of methotrexate, a widely used anticancer drug. The results of the study reveal that relative weight correlates well with the anthropometric parameters, nutritional parameters, and dietary intake and may be used as a marker of nutritional status. When grouped on the basis of relative weights, the undernourished patients revealed a significant ($P < 0.01$) prolongation of biological half-life and a significant ($P < 0.001$) reduction in clearance. Based on these results, relative weight has been proposed as a basis for drug dosage determinations in place of the existing practice of administering antineoplastic drugs on the basis of body surface area or body weight alone. [Cancer Treat Rep 68:727–732, 1984]

Pharmacokinetics is the quantitative study of the metabolic processes of drug absorption, distribution, biotransformation, and elimination (1). Nutritional deficiencies have been shown to alter all of these processes (2–10). There is a remarkable paucity of information on drug disposition and pharmacokinetics in varying degrees for malnutrition. This is especially true for anticancer drugs, which have a low therapeutic index and entail a great risk when used.

Methotrexate (MTX), the well-known antimetabolite of folic acid, is a widely used anticancer drug that inhibits the enzyme dihydrofolate reductase. The clinical usefulness of MTX is limited by significant toxicity. The maximum tolerated dose of MTX in man varies between 80 and 900 mg/m^2 of body surface area (11). It is possible that this wide range in sensitivity is due to variations in pharmacokinetics of MTX, which in turn may be influenced by a variety of factors including nutritional status of the patients.

The present investigation was designed to evaluate the effect of nutritional status of cancer patients on the pharmacokinetics of MTX and to assess the specific role of any critical nutrient or nutrients.

MATERIALS AND METHODS

Patients.—Patients with inoperable carcinomas of head and neck who were not obviously cachectic were studied. All of the patients had normal renal and liver functions and had no evidence of myelosuppression, as detected by clinical tests; none had received prior chemotherapy, and no other drugs were administered for 24 hours prior to and 24 hours following drug administration. Each patient received MTX by iv bolus at a dose of 50 mg/m^2 of body surface area.

Sample collection and analysis.—One day prior to drug administration the clinical blood profile, anthropometric measurements, clinical signs of malnutrition, and diet history were recorded (12). Twelve blood samples were collected from each patient (from the antecubital vein contralateral to that where MTX was given) at various time intervals from 30 minutes to 24 hours after MTX drug administration. The 0-hour sample (ie, prior to drug administration) and 24-hour sample were used for evaluation of the biochemical parameters of nutritional status. The samples were centrifuged at 4000 rpm and the serum was stored at -20°C until use. Total void volume of urine

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was collected at various time intervals for 24 hours. The samples were analyzed within 48 hours for MTX through fluorescence detection (13).

Biochemical analysis.—Standard methods were used for the estimation of clinical parameters. Serum proteins (14), vitamin A (15), and vitamin C (16) were assayed spectrophotometrically.

RESULTS

Clinical examination of the patients revealed that cardiovascular, central nervous, and respiratory systems were normal. The hemogram, serum enzymes (alkaline phosphatase, SGPT, and SGOT), kidney function tests, BUN, plasma creatinine, and electrolytes were all within normal limits.

The parameters of nutritional assessment (ie, anthropometric measurements; plasma levels of albumin) revealed a good correlation with relative weight. Relative weight is a term coined to express the percentage weight deficit in a patient (17):

$$\text{relative weight} = \frac{\text{actual weight}}{\text{desirable weight}} \times 100.$$

Since relative weight proved to be a good indicator of the overall nutritional status of the patient, the patient population was categorized into two groups. Those patients with relative weights of ⩾ 65% (65%–90%) were classified as better-nourished and those with relative weights < 65% (50%–64%) were classified as undernourished.

Of the 15 patients entered in this study, two did not complete it and therefore could not be considered for the analysis. Eight patients were classified as better-nourished and five were classified as undernourished.

Anthropometric Measurements

The pattern and physical state of the body, although genetically determined, are profoundly influenced by diet and consequently by nutritional status. Thus, anthropometric measurements are useful criteria for assessing nutritional status of an individual (12).

The arm (17.7 ± 0.255 cm) and chest (75.4 ± 0.570 cm) circumferences in the undernourished group (fig 1) were significantly lower ($P < 0.005$ and $P < 0.001$, respectively) than those of the better-nourished patients (21.81 ± 0.626 cm and 82.188 ± 1.188 cm, respectively).

Biochemical Profiles

The biochemical markers of nutritional status (fig 2) did not reveal any precise evidence of undernutrition. However, intergroup comparison showed that plasma albumin (5.64 ± 0.35 g/100 ml) and vitamin C (0.49 ± 0.06 mg/100 ml) were higher in the better-nourished population as compared to the albumin (4.85 ± 0.25 g/100 ml) and vitamin C (0.382 ± 0.014 mg/100 ml) in the undernourished group, although the differences were not sta-

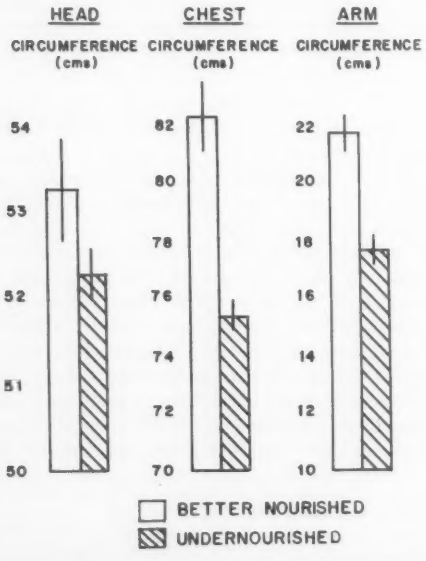

FIGURE 1.—Anthropometric profile.

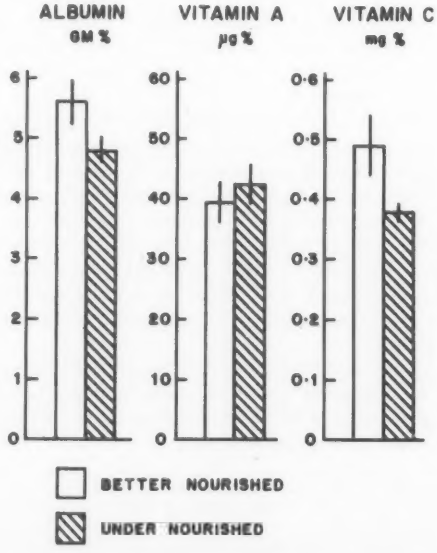

FIGURE 2.—Nutritional profile.

tistically significant. The bromocresol green dye-binding method, used in the present study, has demonstrated values that are consistently in the higher range (18). Vitamin A, however, showed no difference between the two groups.

Clinical Signs

The clinical manifestations of nutritional deficiencies develop over a period of prolonged severe malnutrition. Since most of the patients in the present study were mildly to moderately undernourished, there is no clinical manifestation of any nutrient deficiency. The calf tenderness present in six of our patients reflects a dietary thiamine deficiency (19). Discolored and dyspigmented hair and muddy sclera, observed in some of our patients, are group-II signs, ie, they could be due to a variety of factors including malnutrition and hence do not represent any conclusive evidence (12). No edema was observed in any patient.

Dietary History

Dietary history was collected by the 24-hour recall method (12). Analysis of the major nutrients revealed that the protein and caloric consumption (fig 3) in the undernourished group was considerably lower than that in the better-nourished group.

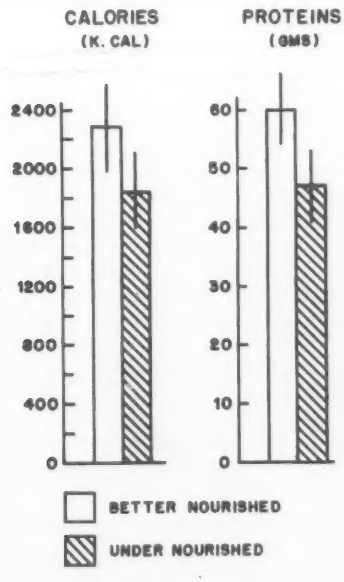

FIGURE 3.—Dietary intake.

Pharmacokinetic Data

The postinjection plasma-concentration time curves showed a biexponential decay when plotted semilogarithmically. Following curve stripping, each phase was subjected to a best fit through multiple regression analysis on a portable computer. The data showed a best fit to the equation

$$C = Ae^{-\alpha t} + Be^{-\beta t},$$

where C is concentration in plasma at time t, α and β are the exponential coefficients during the two phases, and A and B are the contributions of the respective exponential terms at t = 0 (intercepts with ordinate) (19).

A traditional view of the biphasic nature of the postinfusion MTX decay is to assume that there are two lumped body regions that contribute to the decay, a rapidly mobilizable region and a slowly mobilizable region (20).

The distribution (α) and elimination (β) half-lives, apparent volume of distribution (V_0) at time 0 (t_0), pseudodistribution equilibrium volume of distribution (V_D), plasma clearance (Cl), rate constants for intercompartmental drug movement (K_{12}, K_{21}), and elimination rate constant (Kel) were calculated as per the following equations (1).

$$T_{1/2\alpha} = \frac{0.693}{\alpha}$$

$$T_{1/2\beta} = \frac{0.693}{\beta}$$

$$ke = \frac{A + B}{\frac{A}{\alpha} + \frac{B}{\beta}}$$

$$k_{21} = \frac{\alpha\beta}{ke} = \frac{A\beta + B\alpha}{A + B}$$

$$k_{12} = \frac{AB}{(A + B)^2} :: \frac{(\beta - \alpha)^2}{k_{21}}$$

$$V_0 = \frac{\text{Dose}}{A + B}$$

$$V_{D(area)} = \frac{\text{Dose}}{\beta\,(\text{Area under the serum concentration curve from } t = 0 \text{ to } t = \infty)}$$

$$= \frac{\text{Dose}}{\beta\left(\frac{A}{\alpha} + \frac{B}{\beta}\right)}$$

$$\text{Clearance} = \frac{\text{Dose}}{\text{Area under the serum concentration curve from } t = 0 \text{ to } t = \infty}$$

$$= \frac{0.693}{t_{1/2\beta}} \cdot V_{D(area)}$$

$$= Vo.ke.$$

Since at time 0 all drug is present in the central compartment, Vo is also the volume of the central compartment. V_T - the volume of the tissue compartment is, therefore, $(V_D - V_0)$.

The plasma decay curves of the two groups are shown in figure 4. The pharmacokinetic profile of the two groups is graphically represented in figures 5 and 6 and schematically represented in figure 7.

The half-life of MTX in the undernourished group (40.43 ± 9.48 hours) is significantly ($P < 0.01$) prolonged, compared to the better-nourished group (16.63 ± 1.11 hours). In the undernourished group the central compartment volume of 5.882 ± 0.778 L shows a reduction, while the tissue compartment volume of 151.694 ± 75.06 L indicates a definite increase, compared to the better-nourished group with a central compartment volume of 8.20 ± 1.29 L and a tissue compartment volume of 86.99 ± 11.517 L. On the contrary, clearance of 2.22 ± 0.41 ml/minute and elimination constant Kel of 0.4196 ± 0.10/hour are markedly reduced in the undernourished group, compared to a clearance of 4.18 ± 0.29 ml/minute and an elimination constant Kel of 0.7271 ± 0.15/hour in the better-nourished group.

As a corollary to the decreased plasma clearance, total urinary output of MTX (fig 5) in the undernourished group is significantly ($P < 0.05$) reduced.

DISCUSSION

The factors affecting distribution of drugs include body composition, plasma protein and rbc binding, variability in the apparent volume, and hemodynamic status of the patients (19).

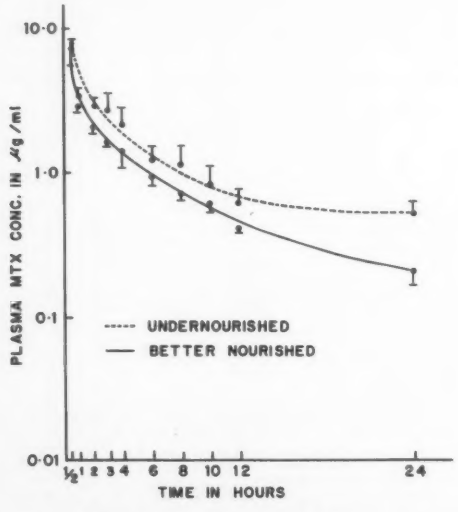

FIGURE 4.—Plasma decay curves of MTX.

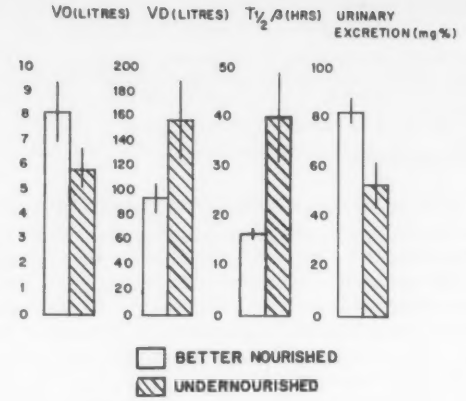

FIGURE 5.—Pharmacokinetic parameters.

Changes in body composition may include changes in total body water, lean body mass, or body fat which will cause a corresponding change in drug distribution. In the undernourished group, a deficit in lean body mass with relative overhydration affecting primarily the extracellular space has been observed (21).

Distribution throughout the extracellular water is important for access of drug molecules to their site of action. Changes in these fluid volumes are most likely to affect the clearance of the drug from the body by altering the distribution. Drugs with increased volume of distribution leave the body slowly and are generally more toxic than when they do not distribute deeply into the body tissues (19).

The increased tissue distribution in the undernourished group of the investigation reported here may be one of

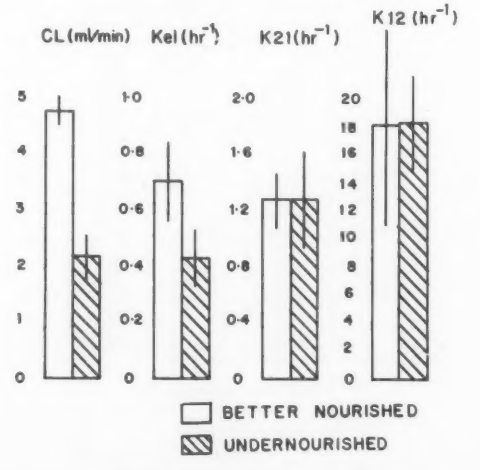

FIGURE 6.—Pharmacokinetic profile.

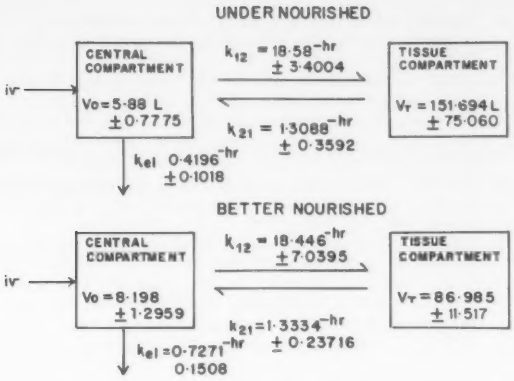

FIGURE 7.—Schematic representation of pharmacokinetic model.

the contributing factors that result in enhanced toxicity when MTX is used in treatment.

Clearance

The impaired plasma clearance in the undernourished group indicates that one or more routes of body clearance are affected. Although many routes of drug excretion are important, only hepatic biotransformation and biliary and renal excretion make important contributions to drug response.

Hepatic Biotransformation

A variety of factors including nutritional status have been demonstrated to have a significant bearing on hepatic detoxification or activation (5). Although metabolism is not a prominent feature of MTX pharmacokinetics (20), it is known that 7-OH-MTX is formed in humans by the action of hepatic aldehyde oxidases. 2,4-Diamino-N^{10}-methyl pteroate results from the action of the intestinal flora on the drug that is excreted into the gut. At late time (> 36 hours), these metabolites may predominate over the parent drug.

In the present investigation, the spectrofluorometric method employed does not distinguish between parent drug and metabolites. However, earlier reports suggest that metabolites have not been detected in plasma or urine at this low-dose regimen prior to 24 hours (22). Thus, changes in hepatic biotransformation capacity, if any, could not have been responsible for the delayed clearance observed in the present investigation.

Renal Excretion

The portion of each dose of MTX that is normally excreted rapidly gains access to the urine by a combination of glomerular filtration and active tubular secretion (23). Biliary excretion also occurs but it appears that enterohepatic circulation greatly reduces the potential fecal excretion (20).

The total 24-hour urinary excretion of MTX in the better-nourished group ranged from 70% to 95% (mean, 82.55% ± 4.66%), while in the undernourished group it ranged from 30% to 70% (mean, 53.39% ± 9.14%). The values for the normal patients are in close agreement with Henderson and co-workers (24), who reported 58%–92% of the dose to be excreted in the urine. Rate constants for intercompartmental exchange (K_{12}, K_{21}) are comparable, but the elimination constant (Kel) from the body is markedly reduced in the undernourished group. This correlates well with the decreased plasma clearance ($P < 0.001$) and decreased urinary excretion ($P < 0.05$). The reduced clearance of the drug from the body could thus be due to an alteration either in the glomerular filtration rate (GFR) or tubular secretion. The most important factor in the variability of drug response due to kidney malfunction is the change in GFR. Elimination half-life is related to GFR and drugs which are primarily eliminated through the kidneys show significant changes in half-life even if impairment is mild (19).

Klahr et al (8,9) have demonstrated an impairment in renal function in undernutrition; mild to moderate impairment in renal function was difficult to detect using BUN or plasma creatinine levels as criteria. In the present study, too, all patients showed normal levels of BUN and plasma creatinine.

Active tubular secretion involves a carrier protein (25). It is possible that in undernutrition the levels of this carrier protein too are low and may result in decreased excretion of the drug. However, since creatinine clearance was not measured in the present study, it is difficult to pinpoint the cause of decreased renal clearance of MTX.

To summarize, in undernutrition, the plasma half-life of MTX is significantly ($P < 0.01$) prolonged, accompanied by increased volume of tissue distribution and a markedly decreased clearance of the drug ($P < 0.001$).

For optimum therapeutic response drug dosage must be individualized, and it has been observed that the response to some drugs is better correlated with the steady-state plasma concentration than to dosage (26). Moreover, plasma monitoring of MTX has been successfully used to predict and prevent MTX toxicity on a high-dose regimen (27). In the present study the dosage used was too low for observation of any toxicity manifestations. However, toxicity may have become apparent with a high-dose regimen.

Since undernutrition has altered the pharmacokinetics of MTX, it deserves to be taken into consideration when the drug dosage is established for a patient. Thus, for better therapeutic response, relative weight could be used as a guide for drug dosage determinations, especially in undernutrition.

REFERENCES

1. KOCH-WESER J, and GREENBLATT DJ. Clinical pharmacokinetics. N Engl J Med 293:702-705, 1975.
2. MAYORAL LG, TRIPATHY K, GARCIA ET, ET AL. Malabsorption in tropics: a second look—the role of protein malnutrition. Am J Clin Nutr 20:866-883, 1967.
3. GOETSCH R. Effect of folate deficiency on the intestinal mucosa on jejunal transport in the rat. J Lab Clin Med 89:1002-1008, 1977.
4. VITERI FE, and ARROYAYE G. Protein calorie malnutrition. *In* Modern Nutrition in Health and Disease, 5th ed (Goodheart RS, and Shils ME, eds). Philadelphia, Lea and Febiger, 1974, pp 604-624.
5. BASU TK, and DICKERSON JWT. Interrelationships of nutrition and the metabolism of drugs. Chem Biol Interact 8:193-206, 1974.
6. CAMPBELL TC, and HAYES JR. Role of nutrition in the drug metabolizing enzyme system. Pharmacol Rev 26:171-197, 1974.
7. ZANNONI VG, and SATO PH. The effect of certain vitamin deficiencies on hepatic drug metabolism. Fed Proc 35:2464-2469, 1976.
8. KLAHR S, and TRIPATHY K. On the evaluation of renal function in malnutrition. Arch Intern Med 118:322-325, 1966.
9. KLAHR S, TRIPATHY K, GARCIA FJ, ET AL. On the nature of the renal concentration defect in malnutrition. Am J Med 43:84-95, 1967.
10. NARANG RK, SAROJ M, and MATHUR VS. Pharmacokinetic study of antipyrine in malnourished children. Am J Clin Nutr 30:1979-1982, 1977.
11. HANSEN HH, SELAWRY OS, HOLLAND JF, ET AL. The variability of individual tolerance to methotrexate in cancer patients. Br J Cancer 25:298-305, 1971.
12. JELLIFFE DB. The Assessment of Nutritional Status of the Community. WHO Monograph Series No. 83, 1966.
13. CHAKRABORTI SG, and BERNSTEIN IA. A simplified fluorometric method for the determination of plasma methotrexate. Clin Chem 15:1157-1161, 1969.
14. BARTHOLOMEW RJ, and DELANEY AM. Spectrophotometric studies and analytical applications of the protein error of some pH indicators. Proc Aust Assoc Clin Biochem 1:64, 1966.
15. NEELD JB, and PEERSON WA. Macro and micro methods for the determination of vit A using trifluoroacetic acid. J Nutr 79:453-462, 1963.
16. LOWRY OH, LOPES JA, and BESSEY OA. Determination of ascorbic acid in small amounts of blood. J Biol Chem 160:609-615, 1945.
17. VAN ITTALIE TB. Assessment of nutritional status. *In* Principles of Internal Medicine, 8th ed (Thorn GW, Adams RD, Braunwald E, et al, eds). Tokyo, McGraw-Hill, Kogakusha, Ltd, 1977, pp 445-449.
18. WEBSTER D, BIGNELL AHC, and ATTWOOD EC. An assessment of the suitability of bromocresol green for the determination of serum albumin. Clin Chim Acta 53:101, 1974.
19. NIAZI S. Text Book of Biopharmaceutics and Clinical Pharmacokinetics. New York, Appleton Century Crofts, 1979.
20. CREASEY WA. Pharmacokinetics. *In* Drug Disposition in Humans—The Basis of Clinical Pharmacology. New York, Oxford University Press, 1979, pp 88-120.
21. VITERI FE, and ARROYAYE G. Protein calorie malnutrition. *In* Modern Nutrition in Health and Disease, 5th ed (Goodheart RS, and Shils ME, eds). Philadelphia, Lea and Febiger 1974, pp 604-624.
22. HUFFMAN DH, SUK HAN WAN, AZARNOFF DL, ET AL. Pharmacokinetics of methotrexate. Clin Pharmacol Ther 14:573-579, 1973.
23. GILMAN AG, GOODMAN LS, and GILMAN A. Anti proliferative agents and drugs used for immunosuppression. *In* The Pharmacological Basis of Therapeutics, 6th ed. New York, MacMillan Publishing Co, 1980, pp 1256-1313.
24. HENDERSON ES, ADAMSON RH, and OLIVERIO VT. The metabolic fate of tritiated methotrexate. II. Absorption and excretion in man. Cancer Res 25:1018-1024, 1965.
25. CHRISTOPHIDIS N, LOUIS WJ, LUCAS I, ET AL. Renal clearance of methotrexate in man during high dose oral and intravenous infusion therapy. Cancer Chemother Pharmacol 6:59-64, 1981.
26. CROWTHER D. Cancer chemotherapy. *In* Treatment of Cancer (Halnan KE, ed). London, Chapman and Hall, 1982, pp 73-101.
27. STOLLER RE, HANDE KR, JACOBS SA, ET AL. Use of plasma pharmacokinetics to predict and prevent methotrexate toxicity. N Engl J Med 297:630-634, 1977.

Use of a Human Tumor Cloning System To Evaluate Analogs of Methotrexate and Mitoxantrone[1,2]

Bernd Lathan,[3,*] Daniel D. Von Hoff,[3] and Edward Elslager[4]

We have utilized a human tumor cloning system to compare the antitumor activity of trimetrexate (TMQ), a new dihydrofolate reductase inhibitor, and ametantrone, a new anthracenedione, with that of analogs already in clinical trial (methotrexate and mitoxantrone). Sixty-nine of 136 tumors plated for the TMQ study and 84 of 228 tumors plated for the ametantrone study were evaluable for drug-sensitivity assays. The overall in vitro response rates (defined as a ≤ 50% survival of tumor colony-forming units) for TMQ were 20% and 23% at 0.1 and 1 μg/ml, respectively; for ametantrone they were 13%, 21%, and 26% at 0.1, 1, and 10 μg/ml, respectively. The overall in vitro activity for both new compounds was similar to that of their clinically used analogs, but TMQ was active in eight of 47 methotrexate-resistant specimens and ametantrone in nine of 62 mitoxantrone-resistant specimens. A comparison of these in vitro results with the results of phase II clinical trials with both drugs should allow an evaluation of the utility of the human tumor cloning system for predicting clinical antitumor activity of analogs of currently available antineoplastic agents. [Cancer Treat Rep 68:733–738, 1984]

Trimetrexate (TMQ) is a new dihydrofolate reductase inhibitor with activity superior to that of the classical antifol methotrexate (MTX) in solid tumors (B16 melanoma, CD8F1 mammary tumor, and colon carcinomas 26 and 38) and L1210 and P388 leukemias in mice (fig 1) (1–3). The compound has not yet undergone phase I trials in man.

Ametantrone has been the third anthracene derivative to complete phase I clinical trials (4,5). It had shown significant antitumor activity against solid tumors (B16 melanoma; CD8F1 mammary tumor; M5076 ovarian carcinoma; and colon carcinomas 76, 36, 38, 06A, and 07A) and leukemias L1210 and P388 in mice (6). In phase I clinical trials, the maximal tolerated dose was 135–180 mg/m^2, with the dose-limiting toxic effect being reversible leukopenia (4). Amentantrone is closely related to mitoxantrone, a compound now undergoing phase II trials (fig 2).

In the development of analogs of clinically useful antineoplastic agents, one important question is whether the analog will have superior antitumor activity in man. This question is not generally answered before phase II clinical trials are completed. In an attempt to evaluate both new compounds earlier, we have utilized a human tumor cloning system (7,8) to compare the cytotoxic activity of TMQ and ametantrone with that of their clinically used analogs and mitoxantrone.

MATERIALS AND METHODS

After informed consent, a total of 280 patients underwent thoracentesis, paracentesis, bone marrow examination, or surgery as part of routine diagnostic workups or therapeutic maneuvers. Specimens, consisting of solid tumor, ascites, bone marrow, and pleural fluid, were sent for cloning in soft agar. Ninety percent of the patients had received prior chemotherapy.

Collection of Cells

Effusions were collected in preservative-free heparinized vacuum bottles, centrifuged at 150 × *g* for 10

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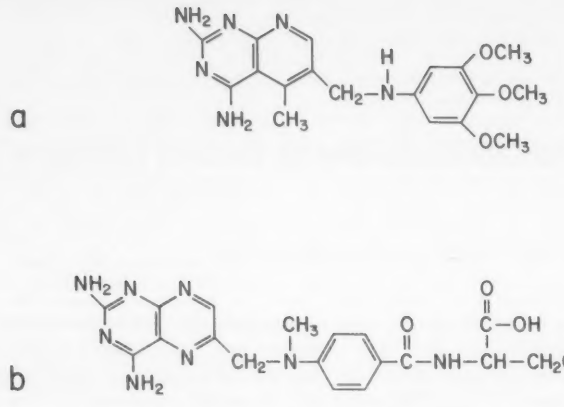

FIGURE 1.—Structures of TMQ (a) and MTX (b).

mins, and washed twice in Hanks' balanced salt solution with 10% heat-inactivated fetal calf serum and 1% penicillin and streptomycin solutions (all materials were from Grand Island Biological Co, NY). Bone marrow specimens were collected in heparinized syringes and processed in the same manner as were the effusions, except that after centrifugation only the buffy coat was removed and processed. Solid tumors removed by biopsy or at surgery were immediately placed in McCoy's 5A medium containing 10% fetal calf serum plus 1% penicillin and streptomycin and were transported from the operating room to the laboratory within 24 hrs, where they were mechanically dissociated by forcing through a wire mesh gauze into Hanks' balanced salt solution plus 10% fetal calf serum. They were then passed through progressively smaller needles and processed in the same manner as were the effusions.

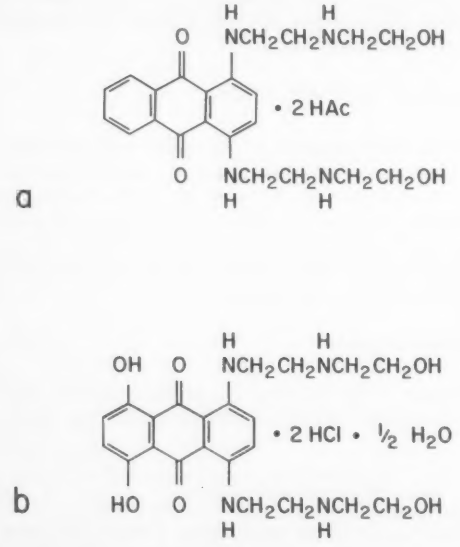

FIGURE 2.—Structures of ametantrone (a) and mitoxantrone (b).

In Vitro Drug Exposure

Stock solutions of the iv formulation of TMQ, MTX, ametantrone, and mitoxantrone were prepared in sterile distilled water and stored at -70°C in aliquots sufficient for individual assays. Subsequent dilutions for incubation with cells were made with sterile distilled water.

For in vitro studies of anticancer agents, it is essential to choose appropriate concentrations of the drugs. In former studies with the human tumor cloning system, the use of about one-tenth of the plasma peak level in patients has been shown to be an appropriate dose for predicting clinical response (9,10). MTX was therefore tested at a final concentration of 0.3 μg/ml based on pharmacokinetic studies showing an average plasma peak concentration of 2.75 μg/ml when 30 mg/m² of MTX has been given to the patients (11). Mitoxantrone was tested at a final concentration of 0.05 μg/ml based on pharmacokinetic studies showing a peak plasma concentration between 0.5 and 0.8 μg/ml when 6 and 12 mg/m² of mitoxantrone has been given to the patients (12).

Pharmacokinetic studies have been performed for the new anthracene derivative ametantrone (4,5).[5] Ametantrone was tolerated at a tenfold higher dose than mitoxantrone in man (13). The peak plasma concentrations were between 4.1 and 15 μg/ml when a dose of 135-180 mg/m² was given to patients (4).[5] We therefore concluded

[5]Kuhn J. Personal communication.

that 1 μg/ml is an appropriate concentration for testing in the human tumor cloning system. We also tested amentrone at concentrations 1 log below and above 1 μg/ml (ie, 0.1, 1.0, and 10 μg/ml).

Since the antifol TMQ has not yet entered phase I clinical trials, no pharmacokinetic data are available in man. In a study administering nonlethal doses of TMQ to dogs, peak plasma concentrations ranged between 0.2 and 5.6 μg/ml (14). Based on this information, TMQ was tested in vitro at the two concentrations of 0.1 and 1.0 μg/ml to blanket this concentration range.

Tumor cell suspensions were transferred to tubes and adjusted to a final concentration of 1.0×10^6 cells/ml in the presence of the appropriate drug dilution or control medium. Cells were incubated with or without drug for 1 hr at 37°C in Hanks' balanced salt solution. The cells were then washed twice with Hanks' balanced salt solution and prepared for culture.

Assay for Tumor Colony-Forming Units (TCFUs)

The culture system utilized in the study has been previously described (7,8,15,16). In brief, cells to be tested were suspended in 0.3% agar in enriched CMRL 1066 medium supplemented with 15% horse serum to yield a final cell concentration of 5×10^5/ml. One milliliter of this mixture was pipetted into each of three 35-ml Petri dishes containing 1 ml of 0.5% agar in enriched McCoy's 5A medium (7,8,15,16) but without conditioned medium. Cultures were incubated at 37°C in 7% CO_2 in humidified air. All assays were performed in triplicate. Colonies (≥ 50 cells) usually appeared in 10–15 days and the number of colonies on control and drug-treated plates was determined with a Bausch and Lomb FASII automatic counter. At least 20 tumor colonies/control plate were required for a drug experiment to be considered evaluable for measurement of drug effect.

Data Analysis

Colony counts of the three plates for a particular drug concentration were averaged to obtain one data point. The standard error of the mean for individual data points averaged 17% of the mean. For determination of sensitivity to a particular drug, the ratio between the number of colonies surviving at each drug concentration and the number of colonies growing in control plates was calculated.

In prior retrospective studies, a ≤ 30% survival of TCFUs was utilized for predicting which patient would respond to a particular conventional chemotherapeutic agent (10,17). This criterion was applied also to investigational drug studies with the cloning system (18,19). A recent prospective clinical trial of the human tumor cloning system revealed the absence of an absolute cutoff point to define in vitro sensitivity (9). In addition, it was shown that a ≤ 50% survival of TCFUs has almost the same predictive value in terms of clinical response as a ≤ 30% survival rate. Therefore, in this study, to make the system more sensitive for new drug screening purposes, a ≤ 50% survival of TCFUs was considered as a definition of activity in vitro.

RESULTS

Trimetrexate

A total of 136 human tumors were placed in culture and had TMQ tested against them. This represented 19 different histologic types of cancer (table 1). Sixty-nine of

TABLE 1.—Summary of culture and sensitivity to TMQ results in human tumor cloning system

Tumor type	No. of cultures which grew ≥ 20 colonies/total No. plated	No. of specimens with ≤ 50% survival of TCFUs	
		0.1 μg/ml of TMQ	1 μg/ml of TMQ
Breast	12/24	0	3
Lung			
Non-small cell	11/24	3	4
Small cell	1/5	0	0
Colon	11/13	5	3
Ovary	8/13	1	1
Renal	8/16	1	0
Melanoma	6/10	2	3
Stomach	3/4	0	1
Lymphoma	3/7	0	0
Pancreas	2/2	1	1
Brain	2/3	1	0
Unknown primary origin	1/4	0	0
Sarcoma	1/1	0	0
Miscellaneous*	0/10		

* Bladder, esophagus, leukemia, liver, myeloma, neuroblastoma, prostate, and thymus.

the 136 specimens (51%) placed in culture formed colonies and were evaluable for drug sensitivity information (ie, growth of ⩾ 20 colonies on control plates). The cloning efficiency for these evaluable specimens varied between 0.004% and 0.63%, with the median cloning efficiency of 0.026%.

Table 1 shows the number of specimens against which 0.1 and 1 μg/ml of TMQ caused a ⩽ 50% survival rate of TCFUs. At both concentrations tested, TMQ demonstrated a ⩽ 50% survival of TCFUs against at least two different patients' tumors for non-small cell cancer of the lung, colon cancer, and melanoma, and against one patients' tumor for ovarian and pancreatic cancers. Against breast cancer, the compound was active only at 1 μg/ml.

Fifty-four of the 136 plated specimens had TMQ (0.1 μg/ml) and MTX (0.3 μg/ml) tested against the same specimens. Figure 3 summarizes the cytotoxic activity of both drugs against the same patients' tumors. Thirty-nine of 54 tumors were resistant to both drugs. Eight specimens showed selective sensitivity to TMQ, whereas four specimens were sensitive only to MTX. TMQ was more often active in vitro than MTX in colon cancer (four vs two specimens), melanoma (two vs one), and ovarian cancer (one vs none).

Ametantrone

Two hundred and twenty-eight human tumors of 18 different histological origins were placed in culture, and ametantrone was tested against them (table 2). Eighty-four of the 228 specimens (37%) formed colonies and were evaluated for drug sensitivity information. The cloning efficiency for the evaluable specimens varied from 0.004% to 0.64% (median, 0.021%). The cytotoxic activity of ametantrone was examined at three different concentrations (ie, 0.1, 1.0, and 10 μg/ml). Table 2 shows the number of specimens in which ametantrone caused a ⩽ 50% survival of TCFUs. There was an increase in the number of sensitive specimens from 11 at 0.1 μg/ml to 18 at 1 μg/ml to 22 at 10 μg/ml of ametantrone. At all three doses, ametantrone had some activity against lymphoma and ovarian, colon, non-small cell lung, brain, and esophagus cancers. At 1 μg/ml, ametantrone caused a ⩽ 50% survival of TCFUs in at least two different patients' tumors for lymphoma, ovarian cancer, non-small cell lung cancer, colon cancer, and melanoma.

Seventy-two of the 228 plated tumors had ametantrone (1.0 μg/ml) and mitoxantrone (0.05 μg/ml) tested against the same tumor. Figure 4 summarizes the cytotoxic activity of both drugs in the same patients' tumors. Fifty-three of 72 specimens were resistant to both compounds. Nine tumors showed selective sensitivity to ametantrone, whereas six specimens were sensitive only to mitoxantrone. Ametantrone was more often active compared to mitoxantrone in lymphoma (five specimens vs one), melanoma (two vs one), esophagus cancer (one vs none), and stomach cancer (one vs none). Mitoxantrone was more often active than ametantrone in lung (two specimens vs none), colon (two vs one), and breast (one vs none) cancers.

DISCUSSION

In retrospective (10,20) and prospective clinical trials (9), the human tumor cloning system has shown some promise for predicting for response or resistance of an individual patient's tumor. In these trials, the assay predicted correctly for resistance in 85%-97% and for true sensitivity in 60%-88% (9,10,20) of the cases. Based on

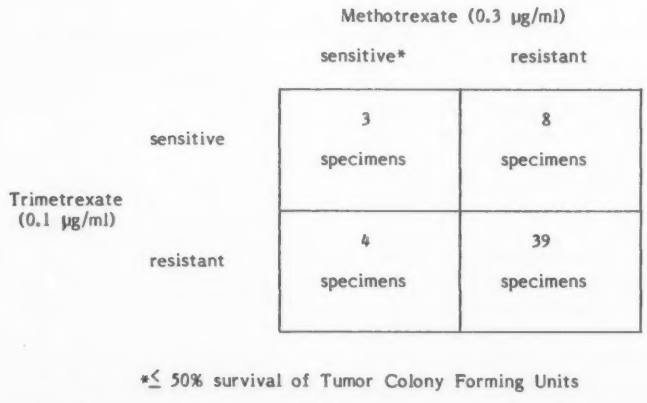

FIGURE 3.—Activity of TMQ and MTX in the same patients' tumors in the human tumor cloning system.

TABLE 2.—Summary of culture and sensitivity to ametantrone results in human tumor cloning system

Tumor type	No. of cultures which grew ≥ 20 colonies/total No. plated	No. of specimens with ≤ 50% survival of TCFUs		
		0.1 μg/ml of ametantrone	1 μg/ml of ametantrone	10 μg/ml of ametantrone
Ovary	15/20	2	3	7
Lung				
Non-small cell	10/27	1	2	2
Small cell	4/11	0	0	0
Colon	12/13	1	2	4
Breast	11/22	1	0	3
Lymphoma	10/77	4	5	3
Stomach	6/11	0	1	0
Pancreas	4/4	0	1	0
Melanoma	4/5	0	2	1
Sarcoma	3/8	0	0	0
Unknown primary origin	2/10	0	0	0
Renal	1/2	0	0	0
Brain	1/2	1	1	1
Esophagus	1/3	1	1	1
Miscellaneous*	0/13			

*Head and neck, leukemia, liver, myeloma, prostate, and thymus.

this experience, the human tumor cloning system might also be utilized for predicting for cytotoxic activity of investigational drugs prior to their entrance in phase II clinical trials. The object of the present study was to investigate if the new drugs TMQ and ametantrone had any cytotoxic activity superior to their clinically useful analogs, and to identify tumor types in which they have their greatest activity.

In this study, TMQ had some cytotoxic activity against non-small cell lung cancer, colon cancer, melanoma, ovarian cancer, and pancreatic cancer. It showed highest activity against colon cancer and non-small cell lung cancer. Weir et al (14) have shown that TMQ is able to penetrate the CNS in dogs. In this connection, it is interesting that TMQ had in vitro cytotoxic activity against one of two human brain tumors.

Diddens et al (21) have investigated the cytotoxic activity of TMQ and MTX in human cell lines resistant to MTX. In two of these cell lines, where resistance to MTX is due to a drug transport defect, TMQ had superior cytotoxic activity. In contrast, cross-resistance to TMQ was observed in MTX-resistant cell lines with normal transport, but increased levels of dihydrofolate reductase (21). This may be explained by different transport systems for TMQ and MTX. The cytotoxicity of MTX may be at least in part due to the metabolism to polyglutamate forms. These polyglutamates can be retained in the absence of extracellular MTX and could cause prolonged cyto-

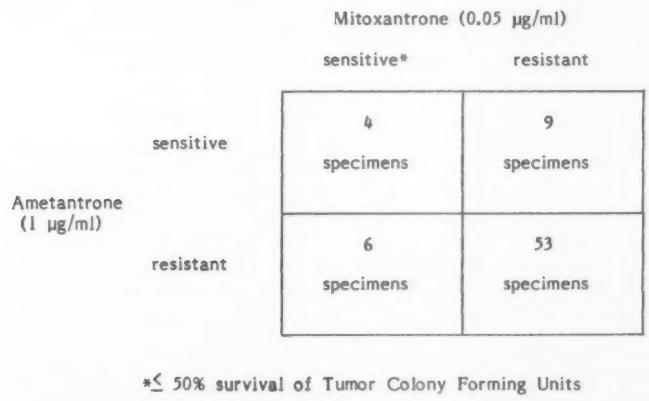

FIGURE 4.—Activity of ametantrone and mitoxantrone in the same patients' tumors in the human tumor cloning system.

toxicity (22-24). Since TMQ lacks a glutamic moiety (fig 1) and cannot be metabolized to polyglutamates, a drug exposure > 1 hr could have been more appropriate for performing comparisons between both antifolates. In view of these findings it is interesting that in our study TMQ had in vitro cytotoxic activity against eight of 47 specimens that were resistant to MTX.

The overall in vitro responses to ametantrone were dose-dependent. At all three concentrations ametantrone was most active in lymphoma, ovarian cancer, colon cancer, and non-small cell cancer of the lung. Ametantrone (1 μg/ml) showed antitumor activity in nine of 62 specimens that were not sensitive in vitro to mitoxantrone (0.05 μg/ml). Of the nine specimens, four were lymphomas. Six of 59 specimens where ametantrone had no in vitro cytotoxic activity were sensitive to mitoxantrone.

From the information obtained in this study, it is clear that TMQ and ametantrone have some degree of cytotoxic activity against different types of human cancers in the human tumor cloning system. When interpreting this information, it is important to remember that most of the patients had received prior chemotherapy before having their tumors tested in this trial. Since most patients in phase II trials will also have had prior chemotherapy, this represents a realistic situation for predicting clinical response of new anticancer agents in early clinical testing. In addition, this study reveals that in some tumors the efficacy of TMQ and ametantrone may be different from that of their clinically used analogs MTX and mitoxantrone.

As clinical phase II trials with TMQ and ametantrone are completed, it will be of great interest to see how the in vitro results presented above compare to the results obtained in phase II trials in patients, and to see if phase II trials carried out in vitro are predictive for in vivo phase II results. If this in vitro phase II methodology looks promising, it could be helpful in screening for analogs of conventional anticancer agents and for selecting tumor types against which these drugs should be used in phase II clinical trials. This would provide maximal utilization of patient resources and decrease the time required for development of a new antineoplastic agent.

REFERENCES

1. BERTINO JR, SAWICKI WL, MOROSON BA, ET AL. 2,4-Diamino-5-methyl(-6-((3,4,5,-trimethoxyanilino)methyl)-quinazoline(TMQ)),a potent non-classical folate antagonist inhibitor. I. Effect on dihydrofolate reductase and growth of rodent tumors in vitro and in vivo. Biochem Pharmacol 28:1983-1987, 1979.
2. BERTINO JR, and SAWICKI WL. Potent inhibitory activity of trimethoxyquine (TMQ), "nonclassical" 2,4-diaminoquinazoline, on mammalian DNA synthesis. Proc Am Assoc Cancer Res and ASCO 18:672, 1977.
3. KAMER BA, EIBL B, CASHMORE AR, ET AL. Efficacy and transport of a new lipid soluble antifol, 2,4-diamino-5-methyl(-6-(3,4,5-trimethoxyanilino)methylquinazoline(TMQ;JB-11)) in methotrexate resistant cells. Proc Am Assoc Cancer Res and ASCO 22:102, 1981.
4. PICCART M, ROZENCZWEIG M, ABELE R, ET AL. Phase I clinical trial with ametantrone (NSC-287513). Eur J Cancer 17:775-779, 1981.
5. LOESCH DM, VON HOFF DD, KUHN J, ET AL. Phase I investigation of ametantrone. Cancer Treat Rep 67:987-991, 1983.
6. CORBETT TH, and GRISWOLD DP. Degree and spectrum of activity of anticancer agents in six colon tumors of mice. Proc Am Assoc Cancer Res and ASCO 20:71, 1979.
7. HAMBURGER AW, and SALMON SE. Primary bioassay of human tumor stem cells. Science 197:461-463, 1977.
8. HAMBURGER AW, and SALMON SE. Primary bioassay of human myeloma stem cells. J Clin Invest 60:846-854, 1977.
9. VON HOFF DD, CLARK GM, STOGDILL BJ, ET AL. Prospective clinical trial of a human tumor cloning system. Cancer Res 43:1926-1931, 1983.
10. VON HOFF DD, CASPER J, BRADLEY E, ET AL. Association between human tumor colony-forming assay results and response of an individual patient's tumor to chemotherapy. Am J Med 70:1027-1032, 1981.
11. ALBERTS DS, and CHEN HSB. Tabular summary of pharmacokinetic parameters relevant to in vitro drug assay. *In* Cloning of Human Tumor Stem Cells (Salmon SE, ed). New York, Alan R. Liss, 1980, pp 351-359.
12. STEWART JA, MCCORMACK JJ, and KRAKOFF IH. Clinical and clinical pharmacologic studies of mitoxantrone. Cancer Treat Rep 66:1327-1331, 1982.
13. VON HOFF DD, POLLARD E, KUHN J, ET AL. Phase I clinical investigation of 1,4-dihydroxy-5,8-bis(((2-hydroxyethyl)amino)ethyl)amino)-9,10 anthracene dione dihydrochloride (NSC 301739), a new anthracenedione. Cancer Res 40:1516-1518, 1980.
14. WEIR EC, CASHMORE AR, DREYER RN, ET AL. Pharmacology and toxicity of a potent "nonclassical" 2,4-diamino quinazoline folate antagonist, trimetrexate in normal dogs. Cancer Res 42:1686-1702, 1982.
15. HAMBURGER AW, SALMON SE, KIM MB, ET AL. Direct cloning of human ovarian carcinoma cells in agar. Cancer Res 38:3438-3444, 1978.
16. VON HOFF DD, CASPER J, BRADLEY E, ET AL. Direct cloning of human neuroblastoma cells in soft agar culture. Cancer Res 40:3591-3597, 1980.
17. VON HOFF DD. Clinical correlations of drug sensitivity in human tumor stem cell assay. Proc Am Assoc Cancer Res and ASCO 21:134, 1980.
18. VON HOFF DD, COLTMAN CA, FORSETH B, ET AL. Activity of 9-10 anthracenedicarboxaldehyde-bis((4,5-dihydro-1*H*-imidazol-2-yl) hydrazone)dihydrochloride (C1 216,942) in a human tumor cloning system. Cancer Chem Pharmacol 6:141-144, 1981.
19. VON HOFF DD, COLTMAN CA, and FORSETH B. Activity of mitoxantrone in a human tumor cloning system. Cancer Res 41:1853-1855, 1981.
20. SALMON SE, ALBERTS DS, MEYSKENS FL, JR, ET AL. Clinical correlations of in vitro drug sensitivity. *In* Cloning of Human Tumor Stem Cells (Salmon SE, ed). New York, Alan R. Liss, 1980, pp 351-359.
21. DIDDENS H, NIETHAMMER D, and JACKSON RC. Patterns of cross-resistance to the antifolate drugs trimetrexate, metoprine, homofolate, and CB 3717 in human lymphoma and osteosarcoma cells resistant to methotrexate. Cancer Res 43:5286-5292, 1983.
22. BAUGH CM, KRUMDIECK CL, and NAIR MG. Polygammaglutamyl metabolites of methotrexate. Biochem Biophys Res Commun 53:27-34, 1973.
23. BALINSKA M, GALIVAN J, and COWARD JK. Efflux of methotrexate and its polyglutamate derivatives from hepatic cells in vitro. Cancer Res 41:2751-2756, 1981.
24. SCHORNAGEL JH, and MCVIE JG. The clinical pharmacology of methotrexate. Cancer Treat Rev 10:53-75, 1983.

Activity and Distribution of Iv and Oral 4-Demethoxydaunorubicin in Murine Experimental Tumors[1,2]

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The antitumor activity of 4-demethoxydaunorubicin (4DDM) compared to its parent compound daunorubicin (DM) was investigated in C57BL/6 mice bearing a T-cell lymphoma, the EL-4, chosen because of its sensitivity to this compound. 4DDM was moderately effective against Lewis lung carcinoma and M5076 ovarian reticulosarcoma tumor systems. Against the EL-4 tumor, after either iv or oral treatment, 4DDM had a good therapeutic effect (survival time in treated mice was almost double that in untreated mice) which was comparable to that of iv doxorubicin. Serum and tissue distribution of 4DDM and its reduced metabolite 4-demethoxydaunorubicinol, given either iv or orally at therapeutic doses to EL-4-bearing mice, was then compared with iv DM using a high-performance liquid chromatography technique with fluorimetric detection. DM seemed to be cleared faster and to a greater extent by metabolism than 4DDM, with half-lives after iv treatment of 23 hrs for 4DDM versus 4.6 hrs for DM. The reduced metabolite in serum amounted to > 100% of the concentration of the native compound for DM and < 20% for 4DDM. By both the iv and oral routes, 4DDM appeared to be concentrated and retained in tissues to a proportionally higher extent than DM, with drug exposure being at least twice as high with correspondingly longer half-lives in almost all tissues investigated, including the tumor. Moreover, this demethoxy analog appeared to be somewhat more selective than DM, since the relative capacity of the tumor tissue to accumulate this compound seemed higher than that of other organs (eg, heart and spleen) reported to be targets of toxicity. Oral administration gave more favorable distribution, resulting in the highest tumor to heart and spleen concentration ratio; this suggests a superiority of this route of administration. [Cancer Treat Rep 68:739–747, 1984]

There is currently great interest in the search for analogs of the anticancer anthracycline antibiotics, daunorubicin (DM) and doxorubicin (DX), which have greater biological activity and less cardiotoxicity than their parent drugs.

4-Demethoxydaunorubicin (4DDM) is a derivative of DM which lacks the methoxyl group in position 4 of the aglycone (1). It has been described as having better properties than the parent compound, ie, greater potency in a spectrum of experimental tumor systems, particularly against experimental leukemias and ascitic tumors of the mouse, although solid tumors generally respond less to iv 4DDM than to DM and DX (2,3).

In vitro 4DDM appears to be much more active in inhibiting exponential growth of HeLa cells and is taken up by mouse embryo fibroblasts to a greater extent than its parent compound (4). The observed increase in biological activity does not parallel an increase in biochemical activity. 4DDM is equivalent to its parent drug in its affinity for DNA and its intercalating capacity into the DNA helix (5–7); it inhibits DNA and RNA synthesis in vitro to about the same extent as DM (4).

The general toxicity of 4DDM seems similar to that of DM although, compared to DM and DX, lower cardiotoxicity was observed in rabbits at iv doses giving equal myelosuppression and in mice and rats at doses corresponding to equal fractions of the LD50 (2,8); myocardiac lesions were found only at high doses (9).

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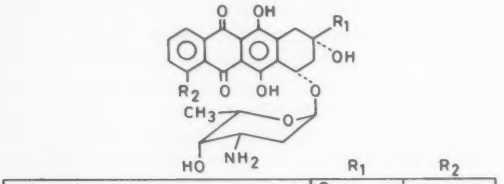
The most relevant property of 4DDM is probably its better oral absorption than that of DM and DX, and this may be accounted for by its higher affinity for lipids, supported by a higher 1-butanol/buffer partition coefficient on removal of the 4-methoxy-group (4).

It has been observed (2,10) that 4DDM, even when given orally, shows a higher therapeutic ratio, exerting significant antitumor activity at nontoxic doses which is definitely superior to that of its parent compound DM and comparable to that of optimal doses of iv DX. The fact that its analogs DX and DM are reported to be inactive and less active, respectively, after oral administration (2,10) suggests that this DM derivative might be superior in clinical use. This is further substantiated by the apparent lack of lesions in cardiac tissue (A. M. Casazza, personal communication) after oral treatment.

A previous study (11) on the distribution of 4DDM in mice analyzed the amount of total fluorescence without distinguishing the parent drug from its fluorescent metabolites. Since 4DDM is a derivative of DM, with metabolite formation that is quantitatively relevant (12,13), and since the activity of anthracycline antibiotics is principally attributable to the parent compound (14,15), separate evaluation of the amounts of native drug and its major metabolites may be important in estimating the level of active drug. A major polar metabolite which, showing the same *Rf* as daunorubicinol (DMol), should be the reduced metabolite of 4DDM, ie, 4-demethoxydaunorubicinol (4DDMol), was detected together with four aglycones (all at very low concentrations) in the liver and possibly in the lungs and stomach (11).

In view of these interesting properties we decided to investigate the pharmacology of this compound. The present report describes a study of the serum and tissue pharmacokinetics of 4DDM and its major metabolite 4DDMol, as compared to DM, after iv or oral therapeutic doses in mice bearing a lymphoma originating from T cells (EL-4 lymphoma) that was selected from among other tumor systems because of its sensitivity to 4DDM.



	R_1	R_2
DOXORUBICIN	$-C(=O)CH_2OH$	OCH_3
DAUNORUBICIN	$-C(=O)CH_3$	OCH_3
DAUNORUBICINOL	$-CH(OH)CH_3$	OCH_3
4-DEMETHOXYDAUNORUBICIN	$-C(=O)CH_3$	H
4-DEMETHOXYDAUNORUBICINOL	$-CH(OH)CH_3$	H

FIGURE 1.—Chemical structures of DX, DM, and 4DDM and their reduced metabolites.

MATERIALS AND METHODS

Animals and Tumors

C57BL/6 male or female mice (body weight, 20 ± 2 g) from Charles River, Italy, were used for these experiments. The tumor systems used were: (*a*) Lewis lung carcinoma (LL) implanted im in male mice (10^5 viable cells/0.1 ml/mouse), which gives rise to macroscopic metastases to the lung; (*b*) M5076 ovarian sarcoma (M5) implanted im in female mice (7.10^5 cells/0.1 ml/mouse), which metastasizes to the liver, uterus, and ovary; and (*c*) thymus-derived T-cell lymphoma EL-4 (obtained from Istituto dei Tumori, Milan, Italy) maintained in ascites form and transplanted ip in female mice (10^6 cells/0.2 ml/mouse) (16).

Drugs

DX, DM, DMol, 4DDM, 4DDMol (fig 1), and the two metabolites found after glycosidic cleavage of DM and 4DDM (daunorubicin aglycone [DM-one] and 4-demethoxydaunorubicin aglycone [4DDM-one]) were kindly supplied by Farmitalia-Carlo Erba, Milan, Italy. The drugs were dissolved in saline for animal treatment and in methanol for the standard solutions for biochemical assay.

Evaluation of Antitumor Activity

In the LL system, the drugs (DX, DM, and 4DDM) were given iv or orally (4DDM) 14 days after im tumor implantation. Ten mice per group were killed on Day 25 after tumor transplant and weights of tumor and lung metastases were recorded. Lung metastases were weighed by dissecting tumor nodules, according to a previously described procedure (17).

In the M5 system, treatment was also given on Day 14 after tumor implantation; ten mice per group were killed on Day 32 after tumor implant and the weights of tumor, liver, ovary, and uterus were recorded. For both LL and M5 tumor systems, mean and median survival times (ST) were calculated in a second group of animals (ten mice for each schedule).

In mice bearing ip EL-4 lymphoma, treatment was given 1 or 3 days after cell inoculum (ten mice per group) and only mean and median ST were calculated.

For all tumor systems, deaths among treated mice occurring before the death of the first control mouse (Day 21 for LL, Day 23 for M5, and Day 11 for EL-4 tumor)

were considered toxic deaths and were not included in the evaluation.

Pharmacokinetic Studies

For pharmacokinetic studies, mice bearing 7-day EL-4 lymphoma were used, ie, at a stage of tumor growth when a solid neoplastic mass is present in the pancreatic region, possibly due to neoplastic transformation of the mesenteric lymph nodes, but ascites has not yet occurred.

Single iv injections of DM and 4DDM were given at doses of 15 and 3 mg/kg, respectively; 4DDM was given orally at a single dose of 7.5 mg/kg. Four animals per point were killed 1, 5, 15, 30, and 60 mins and 3, 6, 24, and 48 hrs after treatment; serum and tissues (tumor, heart, spleen, liver, kidney, and intestine) were removed and immediately frozen at -20°C until analysis.

Analytical Assay

DM, 4DDM, and their 13-hydroxylated metabolites DMol and 4DDMol were quantified by a high-performance liquid chromatography (HPLC) method with fluorimetric detection. Serum samples were extracted with 8 ml of chloroform-isopropanol (1:1) and centrifuged at 4000 rpm; the organic phase was transferred to a fresh tube and evaporated to dryness under vacuum. The residues were redissolved with 100 μl of mobile phase containing DM-one as internal standard for 4DDM and 4DDM as internal standard for DM.

After homogenization (in water), tissue samples were deproteinized with 30 μl of 33% $AgNO_3$, extracted with 8 ml of isopropanol, and then processed as described for extraction for serum.

Extracts (5–20 μl) were injected into the HPLC (Perkin-Elmer Series 3-B) with fluorescence detection (Perkin Elmer 650/10) at an excitation wavelength of 475 nm and an emission of 580 nm. Separation was performed using an isocratic solvent system of water:acetonitrile:0.1 M phosphoric acid (37:37:26) at a flow rate of 1.5 ml/min, using a 25-cm Lichrosorb RP18 (10-μm) column from Merck, Darmstadt, Germany. Recovery was 85% ± 3% and 85% ± 2% for plasma and tissues, respectively, and sensitivity was 5 ng/ml of serum and 20 ng/g of tissue for both DM and 4DDM.

Statistical and Pharmacokinetic Analysis

For evaluation of antitumor activity (tumor and metastases weight; mean ST), statistical significance of the differences between control and treated group values was assessed by Duncan's test. For median ST the Kaplan-Meier method was utilized (18), and the differences were assessed by the regression method (19). Pharmacokinetic parameters in plasma were calculated by the peeling method assuming a simple, two-compartment open model (20); in tissues, the half-life was determined by calculating the significance of the regression using the determinant coefficient r^2 (least-squares method) (21). The area under the concentration versus time curves (AUC) was calculated by trapezoidal integration.

RESULTS

Antitumor Activity

Table 1 shows the antitumor activity of 4DDM and its parent compounds DM and DX against experimental tumors in the mouse after single therapeutic doses. The two solid tumors chosen, im LL and M5, are known to have poor sensitivity to anthracycline antibiotics; the T-cell lymphoma used, EL-4, growing in C57BL/6 mice, seemed to respond to this class of compounds in preliminary experiments. DM and DX were administered iv, since DX is not absorbed by the oral route and DM has considerably less activity when given orally (10).

In this study, DM had practically no effect on LL or M5 in terms of reducing the weight of tumor or metastases, reducing the number of mice with metastases, or prolonging ST. Against EL-4, when treatment was given on Day 1 after tumor inoculum, DM at high doses (18–22 mg/kg iv) resulted in a 50% increase in ST.

DX exerted moderate cytotoxicity, inhibiting tumor or metastases weight (50%–90%) in LL- and M5-bearing mice and reducing the number of mice with metastases. After DX treatment no mice bearing M5 showed metastases to the uterus. A marked increase in lifespan (171% for the median ST and 173% for the mean ST) after DX treatment was observed only in EL-4 lymphoma-bearing mice.

The DM analog 4DDM, like DX, showed limited activity, at least by the iv route, against the LL and M5 tumors but, judging from the percentage of toxic deaths, it seemed more toxic than DX. However, in EL-4 lymphoma-bearing mice treated with 4DDM on Day 1 or 3 after tumor inoculum, a pronounced increase in mean and median ST (often > 100%) with no toxic deaths (no animal dying before controls) was observed after iv or oral 4DDM. In these experimental conditions, 4DDM thus shows therapeutic efficacy practically equivalent to that of iv DX. Moreover, if treatment was given on Day 1 after transplantation, a large percentage of mice (37%–41%) survived > 60 days, at least at the higher iv doses (2.4–3.2 mg/kg).

Serum and Tumor Pharmacokinetics

In view of the sensitivity of the EL-4 lymphoma to iv and oral 4DDM, this tumor system was selected for studies on the comparative pharmacokinetics of optimal doses of DM and its demethoxy analog given iv or orally.

Figure 2 reports the disappearance curve of iv DM, iv

TABLE 1.—Antitumor activity of 4DDM compared to DM and DX (iv or orally) in experimental tumor systems of the mouse*

Tumor (route of implant)	Treatment				% tumor weight inhibition†	% metastasis weight inhibition†				Survival T/C × 100‡		% toxic deaths§
	Drug	Dose (mg/kg)	Route	Day		Lung	Liver	Ovary	Uterus	Mean	Median	
LL (im)	DM	10	Iv	14	29¶	56 (100)	—	—	—	116	123	12.5
	DX	10	Iv	14	47¶	96 (88)**	—	—	—	110	114	18
	4DDM	2.5	Iv	14	51**	93 (75)**	—	—	—	116	108	33
	4DDM	6	Oral	14	8	0 (100)	—	—	—	117	113	44
M5 (im)	DM	10	Iv	14	3	—	16 (100)	15 (67)	45 (67)	119	132	0
	DX	10	Iv	14	12	—	28 (100)¶	75 (14)**	88 (0)**	141	135	0
	4DDM	3	Iv	14	12	—	15 (80)	30 (30)	75 (50)**	133	147	19
	4DDM	7.5	Oral	14	10	—	41 (83)**	0 (67)	81 (67)**	132	131	0
	4DDM	2.5 × 3	Oral	14,15,16	ND	—	ND	ND	ND	131	131	0
EL-4 (ip)	DM	10	Iv	1	—	—	—	—	—	112	ND	0
	DM	14	Iv	1	—	—	—	—	—	131	ND	0
	DM	18	Iv	1	—	—	—	—	—	156¶	ND	0
	DM	22	Iv	1	—	—	—	—	—	150¶	ND	16
	4DDM	1.8	Iv	1	—	—	—	—	—	169 (12)**	ND	0
	4DDM	2.4	Iv	1	—	—	—	—	—	188 (37)**	ND	0
	4DDM	3.2	Iv	1	—	—	—	—	—	222 (41)**	ND	0
	DM	15	Iv	3	—	—	—	—	—	114	121	0
	DX	10	Iv	3	—	—	—	—	—	173**	171**	0
	4DDM	3	Iv	3	—	—	—	—	—	208**	193**	0
	4DDM	7.5	Oral	3	—	—	—	—	—	171**	179**	0

* ND = not determined. Values in parentheses = % of mice with metastases.

†Tumor and metastasis weight inhibition was evaluated on Day 25 for LL and 33 for M5 after tumor transplantation. Tumor weight of controls was 10.3 ± 0.6 g for LL and 6.7 ± 0.5 g for M5. All untreated LL-bearing mice had lung metastases (mean weight, 75.7 ± 14.3 mg). All untreated M5-bearing mice had liver metastases (mean liver weight, 2.3 ± 0.2 g), 40% had ovarian metastases (mean ovary weight, 56.8 ± 29.2 mg), and 60% had uterine metastases (mean uterus weight, 145 ± 55.4 mg).

‡T/C = ratio of treated and untreated survival times. Mean and median ST of untreated mice were 25.6 ± 1.5 and 24 days for LL, 26.2 ± 1.6 and 26 days for M5, and 14.3 ± 0.6 and 14 days for EL-4. Values in parentheses = % of mice surviving > 60 days.

§% of mice that died before the death of 1st control.

¶, ** $P < 0.05$ (¶) and $P < 0.01$ (**) vs controls. For methods of statistical analysis, *see* Materials and Methods section.

4DDM, oral 4DDM, and their metabolites DMol and 4DDMol in serum and tumor of mice bearing 7-day EL-4 lymphoma; at this stage of tumor growth, there is solid neoplastic transformation of the mesenteric lymph nodes in the pancreatic region, preceding the appearance of ascites. In the first part of the curve DM concentrations did not significantly differ in relation to the dose from its demethoxy analog; after 6 hrs, DM was eliminated from serum very fast and was no longer detectable 24 hrs after treatment.

4DDM showed more sustained, prolonged serum levels, its concentrations 48 hrs after iv treatment being still relevant. The elimination half-life calculated according to a two-compartment open model was five times higher than that for DM (23.6 hrs for iv 4DDM and 4.6 hrs for DM; tables 2 and 3).

After oral administration the drug concentrations in serum, though very low, were still in plateau 48 hrs after treatment, suggesting that absorption may possibly continue at this time.

If distribution of DM and 4DDM to the tumor compartment (represented in our conditions by the solid neoplastic mass growing in the pancreatic region) is considered, it appears that the extensive deposit reported for anthracyclines in the nonblood compartment (22–24) results in drug availability in tumor for both drugs about 20 times greater than in serum. Moreover, the demethoxy derivative seems to concentrate in the tumor more than its parent drug, since in the two groups given iv treatment, the total drug exposure in tumor, like serum, as a function of the dose utilized was higher in the 4DDM group than in DM-treated mice. Moreover, whereas DM concentrations in the tumor 24 hrs after treatment were about one-fourth those at peak time, 4DDM iv or orally gave 24-hr concentrations comparable to the peak level, accounting for longer persistence of this compound at the tumor site. Unfortunately, the half-life for tumor could not be calculated because there were too few time points.

Concentrations of the reduced DM metabolite DMol in serum were much higher than the native compound only 60 mins after treatment and were still considerable 48 hrs after treatment, whereas the unmetabolized drug had already disappeared at 24 hrs; the calculated half-life of DMol was 13 hrs versus 4.6 hrs for DM (table 2).

After 4DDM treatment, smaller amounts of its reduced metabolite were observed than the native compound; after oral treatment in particular, levels of native drug were very low in serum, and only traces of 4DDMol were measurable within the sensitivity limits of the method.

DMol levels in the tumor were fairly high, rising gradually up to 24 hrs, when its concentrations were completely superimposable to those of DM, and still remaining equal 48 hrs after treatment.

Compared to DMol, the concentrations of 4DDMol in the tumor relative to serum were proportionally much higher; the reduced metabolite of 4DDM, despite its low levels in serum, accumulated somewhat more in the tumor (as a function of the dose) than did the metabolite of its parent drug.

The metabolites found after glycosidic cleavage of these compounds were quantitatively less important, and the concentrations were too low for proper measurement; the largest amounts were found in the liver and kidney after iv DM and 4DDM.

Distribution to Other Tissues

With regard to the distribution of DM and its demethoxy derivative to heart, spleen, liver, kidney, and intestine (tables 2–4), where peak concentrations were obtained primarily about 30 mins after iv treatment and

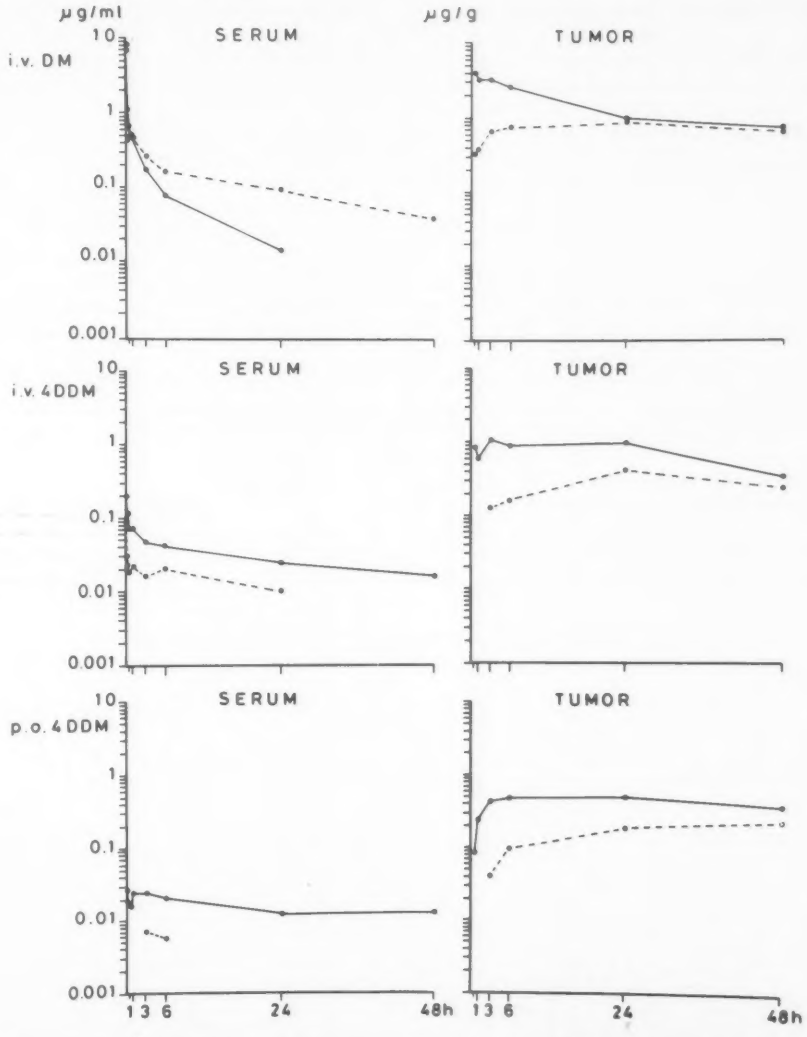


FIGURE 2.—Disappearance curve of iv DM (15 mg/kg) and iv and oral 4DDM (3 and 7.5 mg/kg, respectively) in serum and tumor of mice bearing 7-day EL-4 lymphoma. ●——● = DM or 4DDM; ●---- ●= DMol or 4DDMol.

TABLE 2.—Serum and tissue levels of DM and its metabolite DMol after iv injection of 15 mg/kg to EL-4-bearing mice*

	C_{max}† (μg/ml or g)		AUC (0–48 hrs)‡ (μg/ml or g × mins)		Half-life§ α		Half-life§ β (hrs)		Cl total§ (L/min/kg)		DM-one¶
	DM	DMol	DM	DMol	DM	DMol	DM	DMol	DM	DMol	
Serum	6.9 ± 0.4 (1 min)	8.4 ± 0.9 (1 min)	162 ± 23	361 ± 24	3 mins 41 secs	2 mins 28 secs	4.6	13.9	0.08	0.03	-
Tumor	4.0 ± 0.1 (30 mins)	0.9 ± 0.4 (24 hrs)	4174 ± 802	2170 ± 594	—	—	ND	ND	—	—	-
Heart	18.7 ± 1.2 (30 mins)	3.2 ± 0.2 (180 mins)	7190 ± 1465	5101 ± 889	—	—	7.0	15.7	—	—	-
Spleen	28.5 ± 2.7 (180 mins)	5.8 ± 0.4 (360 mins)	41,279 ± 2882	11,392 ± 809	—	—	12.5	22.6	—	—	-
Liver	27.8 ± 0.5 (30 mins)	8.5 ± 0.8 (180 mins)	8591 ± 780	6903 ± 864	—	—	6.7	11.3	—	—	++
Kidney	72.0 ± 4.5 (30 mins)	33.1 ± 5.3 (30 mins)	18,297 ± 1865	17,837 ± 2482	—	—	6.8	11.2	—	—	++
Intestine	47.3 ± 1.8 (30 mins)	17.0 ± 0.5 (180 mins)	19,860 ± 3375	16,987 ± 2926	—	—	8.8	16.5	—	—	-

* Values = mean ± SE.

†C_{max} = drug concentration at peak. Values in parentheses = time at which peak levels were found.

‡AUC (0–48 hrs) calculated by trapezoidal integration.

§Half-life and Cl total (total clearance) calculated according to the peeling method for serum (ref 20) and least-squares method for tissue (ref 21). ND = not determined because drug levels were still at a plateau 48 hrs after administration.

¶Presence or absence of the aglycone is indicated by ++, +, or -, since the amount of metabolite, when present, was too small for quantification.

TABLE 3.—Serum and tissue levels of 4DDM and its reduced metabolite after iv injection of 3 mg/kg to EL-4-bearing mice*

	C_{max} (μg/ml or g)		AUC (0–48 hrs) (μg/ml or g × mins)		Half-life α	Half-life β (hrs)		Cl total (L/min/kg)	4DDM-one
	4DDM	4DDMol	4DDM	4DDMol	4DDM	4DDM	4DDMol	4DDM	
Serum	0.7 ± 0.05 (1 min)	0.09 ± 0.006 (1 min)	89 ± 4	23 ± 3	4 mins 24 secs	23.6	ND	0.024	-
Tumor	1.0 ± 0.07 (180 mins)	0.4 ± 0.05 (24 hrs)	2214 ± 88	803 ± 101	—	ND	ND	—	-
Heart	5.8 ± 0.5 (30 mins)	0.8 ± 0.04 (360 mins)	2921 ± 253	1448 ± 102	—	10.2	25.0	—	+
Spleen	6.3 ± 1.1 (180 mins)	2.4 ± 0.3 (24 hrs)	10,705 ± 1716	4715 ± 612	—	20.7	ND	—	-
Liver	4.4 ± 0.4 (60 mins)	2.0 ± 0.08 (360 mins)	4062 ± 255	3031 ± 175	—	9.7	15.9	—	++
Kidney	0.6 ± 0.6 (30 mins)	6.1 ± 1.1 (180 mins)	6828 ± 847	8297 ± 1259	—	9.3	16.1	—	++
Intestine	3.9 ± 0.2 (60 mins)	1.5 ± 0.2 (360 mins)	4720 ± 520	2818 ± 399	—	13.1	26.9	—	-

* Values = mean ± SE. *See* legend to table 2.

TABLE 4.—Serum and tissue levels of 4DDM and its reduced metabolite after oral treatment (7.5 mg/kg) in EL-4-bearing mice*

	C_{max} (μg/ml or g)		AUC (0–48 hrs) (μg/ml or g × mins)		β-half-life (hrs)		4DDM-one
	4DDM	4DDMol	4DDM	4DDMol	4DDM	4DDMol	
Serum	0.03 ± 0.009 (1 min)	0.007 ± 0.001 (180 mins)	45 ± 3	ND	ND	ND	-
Tumor	0.5 ± 0.05 (24 hrs)	0.2 ± 0.06 (48 hrs)	1291 ± 222	481 ± 103	ND	ND	-
Heart	1.5 ± 0.2 (180 mins)	0.3 ± 0.04 (360 mins)	1563 ± 161	780 ± 143	14.4	29.7	-
Spleen	1.8 ± 0.3 (180 mins)	1.3 ± 0.6 (24 hrs)	3918 ± 1057	2004 ± 617	ND	ND	-
Liver	2.9 ± 0.3 (180 mins)	0.9 ± 0.1 (360 mins)	2907 ± 346	1568 ± 239	11.8	32.5	++
Kidney	4.9 ± 0.3 (360 mins)	3.6 ± 0.3 (360 mins)	5493 ± 607	7173 ± 1331	14.6	60.4	-
Intestine	64.6 ± 8.3 (30 mins)	1.4 ± 0.4 (180 mins)	23,975 ± 4407	2281 ± 401	6.6	36.5	+

* Values = mean ± SE. *See* legend to table 2.

180 mins after oral treatment, it was observed that iv DM and 4DDM accumulated in the kidney to peak levels 1.5 to three times those in the other organs. Total drug exposure and persistence were significantly higher in the spleen (two to four times higher AUC and half-life almost twice that in the kidney).

For at least 6 hours after oral treatment, 4DDM gave five to ten times higher concentrations in the intestine than in the other organs. In terms of peak or AUC level the kidney was the tissue accumulating the most compound and the spleen did not differ significantly from the other tissues. The high 4DDM levels in the intestine together with its prolonged plateau in serum are compatible with slow, long-lasting absorption.

In general, 4DDM remained in tissues longer than DM after either iv or oral treatment, as already noted. However, compared to the native drug, the reduced metabolites of 4DDM (iv or oral) or of DM were present in high amounts (often > 50% of the concentrations of the native drug) and usually persisted longer in all tissues investigated (longer half-life), suggesting that uptake and retention of these metabolites in tissues are highly relevant, despite the different serum levels.

In this regard it is noteworthy that for both DM and 4DDM, the availability (in terms of AUC) of these metabolites in the kidney was equal to or higher than that of the parent drug.

Aglycones of DM and 4DDM were also found, though at concentrations too low for quantitative measurement in the liver, kidney, and possibly in the intestine after oral treatment.

Tumor to Tissue Distribution Ratios

To estimate the selectivity of the different treatments, the relative distribution of DM and iv or oral 4DDM to organs that are targets of activity and toxicity was compared in terms of tumor to tissue concentration ratios (table 5).

In every tissue, DM gave the lowest ratios of either C_{max} or AUC values. Conversely, the highest tumor to tissue concentration ratios observed for 4DDM indicate that this compound is taken up better than DM in the tumor, more so than in other organs; an exception is obviously the intestine after oral treatment, where the very high local concentrations of 4DDM make the ratios very low.

In the heart, in particular, and in the spleen, although to a lower extent, the relative distribution of 4DDM compared to that of DM seems lower, as accounted for by the higher tumor to heart and spleen concentration ratios. However, the most favorable distributive ratio was after oral 4DDM, which gave tumor to heart and spleen concentration ratios that were twice those after DM and 1.5 times higher than after iv 4DDM, suggesting better selectivity of the oral over the iv schedule.

DISCUSSION

The findings described in the present report indicate that removal of the C4-methoxy group in the aglycone moiety of the DM molecule substantially changes its pharmacological behavior compared to its parent compound. DM as a single iv dose was practically ineffective against solid LL and M5 tumors and the EL-4 lymphoma. Conversely, its demethoxy analog 4DDM, which showed limited activity against the solid tumors, markedly prolonged the ST of mice bearing EL-4 lymphoma (about 80% longer than with DM and about 20% longer than with DX).

The fact that 4DDM displays good antitumor effect compared to DX also when given orally is a further point

TABLE 5.—Tumor/tissue concentration ratio of DM and iv or oral 4DMM*

	DM		Iv 4DDM		Oral 4DDM	
	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC
Heart						
PD	0.22	0.58	0.18	0.76	0.33	0.83
M	0.27	0.43	0.52	0.55	0.83	0.62
Spleen						
PD	0.14	0.10	0.17	0.21	0.28	0.33
M	0.15	0.19	0.17	0.17	0.19	0.24
Liver						
PD	0.15	0.49	0.25	0.55	0.17	0.44
M	0.10	0.31	0.20	0.26	0.28	0.31
Kidney						
PD	0.06	0.23	0.10	0.32	0.10	0.24
M	0.03	0.12	0.07	0.10	0.07	0.07
Intestine						
PD	0.09	0.21	0.27	0.47	0.01	0.05
M	0.05	0.13	0.28	0.28	0.17	0.21

* Values = ratios between the C_{max} and AUC values shown in tables 2–4. PD = parent drug, M = reduced metabolite.

in favor of the therapeutic interest of this compound.

With regard to the pharmacokinetic pattern of DM and its analog, our results confirm the rapid clearance of anthracyclines from plasma and their considerably extensive deposit in tissues, as already described in previous studies (22-24). These drugs tend to distribute to the tissue compartment very rapidly (the distributive phase has a half-life of 3-4 mins) and to a very large extent; the AUC values, which express drug exposure in time, are one or two orders of magnitude greater than in serum.

Significant differences were noted in the pharmacokinetics of DM and 4DDM in mice bearing EL-4 lymphoma given the same iv or oral doses utilized for evaluation of the effect. In serum, after a first distributive phase similar for both compounds, DM seemed to be cleared very rapidly by metabolism, whereas 4DDM persisted in the circulation much longer, with an elimination half-life for the iv route of 23.6 hrs versus 4.6 hrs for DM. By the oral route, serum levels, although very low, were still in plateau 48 hrs after treatment. Whether this observation means that the compound was still being absorbed is a matter of speculation.

After both iv and oral administration, 4DDM appeared to be concentrated and retained in tissues in proportionally higher amounts than DM, the tissue to serum peak concentration ratios being at least twice as high with correspondingly longer half-lives. This finding is in good agreement with the observed greater uptake of this demethoxy derivative of DM by mouse embryo fibroblasts (4) and with other observations (11).

The fact that by the oral route also, despite low serum levels, there was extensive deposition of 4DDM in tumor and tissues suggests that absorption by the oral route, although slow, is quantitatively relevant.

The high, lasting accumulation of 4DDM in tissues is compatible with a continuous uptake in tissues and slow release. Its observed higher affinity than that of DM for lipids (4) might account for this enhanced tissue accumulation as well as for the better oral absorption of 4DDM.

In tumor tissue in particular, DM concentrations started to decline soon after treatment, although slowly, but no disappearance was evident for 4DDM as long as 24 hrs after iv or oral administration. This longer retention of the demethoxy analog at the tumor site, together with its described higher potency (1,2), is likely to contribute to its better therapeutic efficacy than DM.

The very swift clearance of DM from serum appears to be ascribable to rapid and quantitatively relevant biotransformation to its main metabolic product, DMol, by means of cytoplasmic aldoketoreductase, as already reported by other investigators (12,13). Indeed, whereas DM had already disappeared from the circulation 24 hrs after treatment, DMol was still detectable in serum after 48 hrs.

Conversely, much smaller amounts of reduced metabolite amounting to no more than 20% of the concentrations of native compound were found in serum of 4DDM-treated mice, particularly after oral administration, indicating that 4DDM undergoes less carbonyl reduction to 4′-demethoxy DMol than DM.

In tissues, particularly the tumor, DMol reached the same high level as the native drug 24 hrs after treatment, and was present in the other tissues in concentrations amounting to 28%-50% of those of the native drug. Among the tissues investigated, the kidney seems a selective site of drug accumulation, possibly in relation to renal excretion, for either DM or 4DDM, and even more for their reduced metabolites; at this site, the metabolites reach even higher levels than the unchanged drug, at least for 4DDM.

As to whether the differences in pharmacokinetic pattern and metabolic rate observed for the two analogs are involved in differences in response, since the reduced metabolites of anthracycline antibiotics are reportedly much less active in vitro than the parent drugs (14,15), the longer persistence in the body (particularly in the tumor) and the reduced biotransformation of 4DDM as compared to DM may well have some relevance in terms of activity, also in view of the markedly higher cytotoxicity displayed by 4DDM versus DM in vitro (4).

Our findings also suggest a somewhat better selectivity of this DM analog, since the tumor tissue seems to accumulate this compound more than other organs like heart and spleen, reportedly targets of toxicity (25,26) and/or sites of selective accumulation of anthracycline antibiotics (25,27). After oral administration, the distribution ratio of 4DDM is even more favorable, thus suggesting a superiority of this route, which is in good agreement with previous observations (2,10).

It is tempting to speculate that this different distribution, resulting in lower drug concentrations in the heart and spleen than in tumor tissue, is a factor contributing to the better therapeutic effect of 4DDM than DM in EL-4 lymphoma-bearing mice and that toxicity might be lower with equal antitumor activity when the drug is given orally. If this point could be proved and if the activity of oral 4DDM were demonstrated for other tumor systems, oral administration could become the route of choice for this anthracycline analog and its interest in clinical practice should greatly increase.

REFERENCES

1. ARCAMONE F, BERNARDI L, GIARDINO P, ET AL. Synthesis and antitumor activity of 4-demethoxydaunorubicin, 4-demethoxy-7, 9-diepidaunorubicin, and their β anomers. Cancer Treat Rep 60:829-834, 1976.
2. CASAZZA AM. Experimental evaluation of anthracycline analogs. Cancer Treat Rep 63:835-844, 1979.
3. CASAZZA AM, PRATESI G, GIULIANI F, ET AL. Antileukemic activity of 4-demethoxydaunorubicin in mice. Tumori 66:549-564, 1980.
4. SUPINO R, NECCO A, DASDIA T, ET AL. Relationship between effects on nucleic acid synthesis in cell cultures and cytotoxicity of 4-de-

methoxy derivatives of daunorubicin and adriamycin. Cancer Res 37:4523-4528, 1977.
5. DI MARCO A, CASAZZA AM, GAMBETTA R, ET AL. Relationship between activity and amino sugar stereochemistry of daunorubicin and adriamycin derivatives. Cancer Res 36:1962-1966, 1976.
6. ZUNINO F, GAMBETTA R, DI MARCO A, ET AL. Effects of stereochemical configuration on the interaction of some daunomycin derivatives with DNA. Biochem Biophys Res Commun 69:744-750, 1976.
7. PLUMBRIDGE TW, and BROWN JR. Studies on the mode of interaction of 4'-epiadriamycin and 4-demethoxydaunomycin with DNA. Biochem Pharmacol 27:1881-1882, 1978.
8. CASAZZA AM, DI MARCO A, BONADONNA G, ET AL. Effects of modifications in position 4 of the chromophore or in position 4' of the aminosugar, on the antitumor activity and toxicity of daunorubicin and doxorubicin. *In* Anthracyclines: Current Status and New Developments (Crooke ST, and Reich SD, eds). New York, Academic Press, 1980, pp 403-430.
9. ZANON PL, LAMBERTENGHI-DELILIERS G, POZZOLI EF, ET AL. Selective mitochondrial alterations induced by a single dose of daunorubicin or 4-demethoxydaunorubicin in mouse ventricular myocardium. Tumori 66:27-34, 1980.
10. DI MARCO A, CASAZZA AM, and PRATESI G. Antitumor activity of 4-demethoxydaunorubicin administered orally. Cancer Treat Rep 61:893-894, 1977.
11. FORMELLI F, CASAZZA AM, DI MARCO A, ET AL. Fluorescence assay of tissue distribution of 4-demethoxydaunorubicin and 4-demethoxydoxorubicin in mice bearing solid tumors. Cancer Chemother Pharmacol 3:261-269, 1979.
12. FELSTED RL, GEE M, and BACHUR NR. Rat liver daunorubicin reductase. An aldo-keto reductase. J Biol Chem 249:3672-3679, 1974.
13. BACHUR NR, and HUFFMAN DH. Daunorubicin metabolism: estimation of daunorubicin reductase. Br J Pharmacol 43:828-833, 1971.
14. BACHUR NR. Biochemical pharmacology of the anthracycline antibiotics. *In* Cancer Chemotherapy (Sartorelli AC, ed). Washington, American Chemical Society, 1976, pp 58-70.
15. BERAN M, ANDERSSON B, EKSBORG S, ET AL. Comparative studies on the in vitro killing of human normal and leukemic clonogenic cells (CFUc) by daunorubicin, daunorubicinol and daunorubicin-DNA complex. Cancer Chemother Pharmacol 2:19-24, 1979.
16. OLD LJ, BOYSE EA, and STOCKERT E. The G (Gross) leukemia antigen. Cancer Res 25:813-819, 1965.
17. FRANCHI G, and GARATTINI S. Selective chemotherapy of cancer metastases with Triton WR 1339. Eur J Cancer 7:579-580, 1971.
18. KAPLAN EL, and MEIER P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958.
19. ROCCHETTI M, and RECCHIA M. SPBS: statistical programs for biological sciences. Minicomputer software for applying routine biostatistical methods. Comput Programs Biomed 14:7-20, 1982.
20. GIBALDI M, and PERRIER D. Pharmacokinetics. New York, Marcel Dekker, 1975, pp 281-292.
21. SNEDECOR GW, and COCHRAN WG. Statistical Methods, 6th ed, Ames, The Iowa State University Press, 1967.
22. WILKINSON PM, and MAWER GE. The persistence of adriamycin in man and rat. Br J Clin Pharmacol 1:241-247, 1974.
23. YESAIR DW, SCHWARTZBACH E, SHUCK D, ET AL. Comparative pharmacokinetics of daunomycin and adriamycin in several animal species. Cancer Res 32:1177-1183, 1972.
24. BACHUR NR, HILDEBRAND RC, and JAENKE RS. Adriamycin and daunorubicin disposition in the rabbit. J Pharmacol Exp Ther 191:331-340, 1974.
25. SCHWARTZ HS, and KANTER PM. Cell interactions: determinants of selective toxicity of adriamycin (NSC-123127) and daunorubicin (NSC-82151). Cancer Chemother Rep Part 3, vol 6(2):107-114, 1975.
26. YOUNG DM. Pathologic effects of adriamycin (NSC-123127) in experimental systems. Cancer Chemother Rep Part 3, vol 6(2):159-175, 1975.
27. DONELLI MG, MARTINI A, COLOMBO T, ET AL. Heart levels of adriamycin in normal and tumor-bearing mice. Eur J Cancer 12:913-923, 1976.

Amplified Pulmonary Metastases of a Rat Rhabdomyosarcoma in Response to Nitrosourea Treatment[1]

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Repeated observations in our laboratory show that the chloroethylnitrosourea of cysteamine (CNCC) induces slowed tumor growth rate and decreased lymph node metastasis in rats bearing a rhabdomyosarcoma but concomitantly enhances metastatic dissemination in the lung. Tumors obtained by sc graft of tumor cells, in syngeneic rats, gave a reproducible pattern of metastases at nodal and pulmonary sites after a 60–80-day period. CNCC was administered orally at a dose of 50 mg/kg once a week for 5 weeks beginning at the time of tumor appearance. Forty-five of 46 CNCC-treated rats had lung metastases with 95 (± 9.7) nodules; in the control group 29 of 41 rats had lung metastases with 7 (± 1.5) nodules. This amplifying effect was found after treatment with two other nitrosoureas (chlorozotocin and hydroxyethylchloroethylnitrosourea) but not with cyclophosphamide and methotrexate. Lung metastatic amplification was also observed after treatment of the 13762 mammary adenocarcinoma in Fischer rats and treatment of nickel-induced soft tissue tumor. Several hypotheses have been proposed. The dissociated effect of nitrosourea on local tumor, lymph nodes, and pulmonary metastases does not support the concept of systemic immunosuppression as the main mechanism of this phenomenon, but a decrease of local immunological defenses exerted by NK cells, for example, could be possible. Alternatively, a direct effect of the drug on lung tissue, especially lesions of endothelial tissue, could be responsible for the observed effect. Nitrosourea treatment of rats after surgical excision of the tumor, as adjuvant chemotherapy, was responsible for an amplification effect in association with local recurrences. From this fact we hypothesized that nitrosourea treatment could modify the equilibrium of cell subpopulations in the tumor by selecting highly metastatic drug-resistant variants. Although the mechanism of the amplifying effect of nitrosoureas has not been elucidated, our study shows a possible risk in the use of these drugs for inductive or adjuvant chemotherapy. [Cancer Treat Rep 68:749–758, 1984]

Nitrosoureas are an important category of oncostatic agents and have earned an undeniable place in the treatment of human malignancies. Goldin (1) reported the antitumor activity of these drugs against a series of animal tumor models including P388 and L1210 leukemias, B16 melanoma, colon carcinoma 38, Lewis lung carcinoma, and CD8F1 mammary tumor, and against the human tumor xenograft models CX-1 colon tumor, LX-1 lung tumor, and MX-1 mammary tumor implanted in the subrenal capsule. More recently, new chloroethyl derivatives have been synthesized with the goal of finding compounds that have different antitumor spectra and/or less hematologic toxicity. Imbach et al (2) have studied the pharmacology, cytotoxicity, and antitumor activities of the chloroethyl derivatives against a panel of murine tumors. The antitumor effect was generally evaluated in terms of the percent increase in life span. The question of metastases was never specifically addressed. Some authors have previously reported the increase of pulmonary metastases after chemotherapy or radiotherapy. For example, Steel and Adams (3), Milas et al (4), and Van Putten et al (5) obtained an amplification of artificial

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pulmonary metastases following an iv injection of tumor cells by pretreating the recipient mice with cyclophosphamide (CY). Although these experiments dealt with an artificial situation where treatment was given before the iv injection of tumor cells, such a phenomenon of lung metastases amplification has also been described in the case of spontaneous metastases of a transplanted tumor. Moore and Dixon (6) showed a greater dissemination of nodal and pulmonary metastases in rats treated with a combination of ^{60}Co irradiation and CY as compared to other groups of rats.

In our laboratory we have observed that the chloroethylnitrosourea of cysteamine (CNCC) has an enhancing effect on lung metastatic dissemination of a rat rhabdomyosarcoma, while it simultaneously slows the tumor growth rate and decreased lymph node metastases.

The studies reported in this paper were conducted on a transplanted metastatic tumor of the rat. The primary tumor had been induced by im injection of colloidal nickel. The tumor was set in culture and tumor cells were grafted in syngeneic recipient rats. A palpable tumor appeared after an 8-day period in all rats and the tumor growth rate could be measured. The tumor metastasized at the nodal and pulmonary sites. The median survival time was 70 days.

We sought to explore the different parameters and the experimental conditions of metastatic amplification. A more general validation of this phenomenon was approached by the use of two other nitrosoureas compared with two drugs belonging to different families of antitumor agents. Furthermore, we have tried to see if similar paradoxical effects of the drug could be reproduced in another metastatic transplantable tumor of the Fischer rat, in a nonmetastatic tumor of the mouse, and in a nickel-induced tumor of the WAG rat.

MATERIALS AND METHODS

Animals

Female WAG rats, Fischer rats, and C57BL/6 mice were used throughout this investigation as syngeneic recipients of the 9-4/0 rhabdomyosarcoma (WAG), the 13762 mammary adenocarcinoma (Fischer), and the Mc B6-1 fibrosarcoma (C57BL/6). All of the animals were bred at the Institut de Recherches Scientifiques sur le Cancer, used at 10–12 weeks of age, and maintained in specific-pathogen-free conditions.

Tumors

Rhabdomyosarcoma was induced in this laboratory by an im injection of 20 mg of colloidal nickel (Prolabo, France) in the thigh of a WAG rat (7). The tumor was removed and dissociated in a 0.25% trypsin solution. The cells were plated and maintained in a continuous line (designated 9-4/0) in Dulbecco's modified Eagle's medium (Grand Island Biological Co, NY) supplemented with 10% heat-inactivated fetal calf serum (Flow Laboratory, United Kingdom). A 1% antibiotic solution of streptomycin-penicillin (5×10^3 units/ml) was added. Cells were removed by trypsinization from subconfluent cultures. Only suspensions showing > 90% viability by trypan blue exclusion were used. A suspension of 5×10^4 cells in 0.1 ml of medium was injected sc on the flank of rats midway between the inguinal and axillary areas. The 13762 rat mammary adenocarcinoma originally induced with 7,12-dimethylbenzanthracene by Segaloff (8) was maintained by serial transplantation in Fischer rats. A single 2-mm^3 piece of 13762 was implanted sc on the flank of rats with a trochar. The Mc B6-1 murine fibrosarcoma was originally induced by sc injection of methylcholanthrene in this laboratory and maintained by serial in vivo passages on syngeneic C57BL/6 mice. Tumor cells were plated in culture as described previously (9). Tumors were induced by the sc injection of 1×10^4 cultivated cells in the flank of syngeneic recipient mice.

In addition, nickel-induced tumors were obtained at the site of im injection of nickel in WAG rats as described above. Tumors appeared 6–8 months after injection. Rats were separated into two groups in order of tumor appearance and treated with chlorozotocin (CZT) or water (controls) by weekly injections for 5 weeks.

The animals were examined once a week after tumor induction. Following the appearance of palpable tumors, these were measured (the average of 2 diameters) once a week until death. The appearance of palpable metastases at the inguinal and axillary lymph nodes was noted during tumor development. Moribund animals were killed and examined for metastatic invasion. Metastases, especially in the lung, were confirmed by histological examination.

Drugs and Protocols

The CNCC (chloro-2 ethyl)-2*N*-nitroso-*N*-carbamoyl-*N*,*N*-cysteamine) used was the 1604 RB produced by the Roger Bellon Laboratory (Neuilly-sur-Seine, France); this drug, which is not water-soluble, was suspended in 0.5 ml of paraffin oil (Merck) at a dilution of 20 mg/ml and sonicated. Special caution was taken to avoid excessive heating. This suspension was administered to rats by gavage once a week and the first dose was given 8 days after the sc graft of 5×10^4 tumor cells. Usually, rats received weekly doses of 50 mg/kg of body weight for 5 weeks. Two other protocols were used: a single dose of 90 mg/kg or three weekly doses of 50 mg/kg. Control groups received paraffin oil by the same route and with the same protocols. In pretreatment experiments, rats received five doses of 50 mg of CNCC suspended in paraffin oil, weekly for 5 weeks. After an 8-day period following the last ingestion, the tumor cells were grafted sc. For post-

operative treatment experiments, the tumors were excised at 15 mm in diameter and rats were treated 8 days after surgery at a dose of 50 mg/kg (weekly ingestions for 5 weeks).

CZT was provided by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). It was diluted in sterile water and injected ip at a weekly dose of 10 mg/kg, corresponding to the maximal tolerated dose. Hydroxyethylchloroethylnitrosourea was supplied by Dr. Eisenbrand (Heidelberg, W Germany), dissolved in sterile water, and injected at a dose of 5 mg/kg. For these two drugs, HECNU and CZT, the first injection was given 8 days after the tumor graft and repeated weekly five times.

CY (Endoxan Asta 500, Lucien-Lacombe Laboratories, France) was diluted in sterile water and injected weekly at a dose of 50 mg/kg ip The sodium salt of methotrexate (MTX; Lederle Laboratories) was diluted in sterile water and injected three times per week at a dose of 0.4 mg/kg.

Previous experiments performed by Dr. Maral and confirmed in our laboratory had shown that the doses and the adopted protocols corresponded to the maximal doses tolerated by animals, with optimal antitumor effect. All of the drugs were employed immediately after their preparation. Experiments were always reproduced several times. Tables and figures give typical results.

Graft of CZT-Treated Lung Metastatic Tissue

Pulmonary nodules were cut from lungs of rats after development and dissemination of a primary tumor treated with CZT (or H_2O controls). The nodules were dissected and grafted sc to a new series of syngeneic rats. Tumors grew, and autopsies at Day 70 made it possible to count lung nodules.

Histological Examination

Lungs were excised at autopsy, totally fixed in Bouin's solution, and then embedded in paraffin. Sections (3-μm) were prepared and stained with hematoxylin-eosin-safranine and occasionally with reticulin, periodic acid-Schiff (PAS), or Mallory's phosphotungstic acid-hematoxylin (PTHA).

Statistical Analysis

Means of survival times and tumor sizes were calculated as arithmetical means per group of rats identically treated, and standard deviations were determined. Statistical significance of the differences was analyzed by Student's t test. Mean numbers of lung metastatic nodules and standard deviations were calculated, and statistical significance was evaluated by the nonparametric Wilcoxon test (10).

RESULTS

Pattern of Metastatic Dissemination of the 9-4/0 Rhabdomyosarcoma in Nontreated Rats

The invasion of inguinal lymph nodes was observed first in all rats, while the tumor reached a mean diameter of 30 mm corresponding to a 50–60-day tumor growth period. The invasion of axillary nodes was observed later and affected 70% of the rats. Autopsies revealed that the lumbaraortic nodes were frequently metastatic (90% of rats). Lungs were generally invaded; the nodule count at the surface of lungs varied between 1 and 15 (mean, 8 ± 2). The invasion of mediastinal nonpulmonary tissue was frequent (60%) and, with the invasion of lungs, caused a respiratory stress which was the major cause of death. The survival time of tumor-bearing rats varied between 65 and 80 days.

Effect of CNCC Treatment on Tumor Growth and Metastatic Dissemination of the Rhabdomyosarcoma

As a consequence of CNCC treatment, we observed a 30% body weight loss and a slowing of tumor growth rate (fig 1). This slow growth of the tumor was not correlated to the weight loss, as shown by examination case by case. In general, the survival time was shortened, and CNCC-treated rats died in a state of respiratory stress. The autopsy examination showed a considerable invasion of lungs by tumor tissue. The mean nodule count was clearly

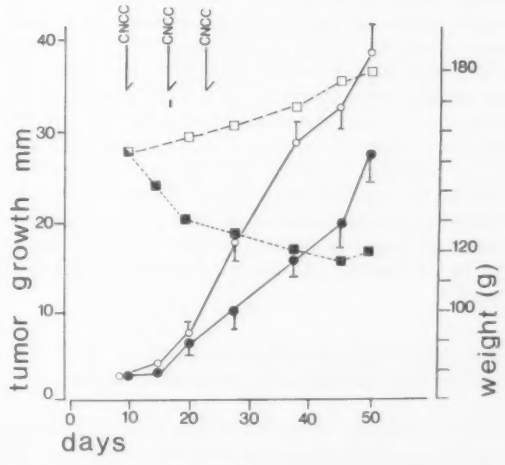

FIGURE 1.—Tumor growth rate of the rhabdomyosarcoma (mean of diameters) in rats treated with oil (O———O) or with CNCC in oil (●———●). Effect of treatments on the mean body weight of rats treated with oil (□———□) or with CNCC in oil (■———■). Rats received 3 weekly doses of drug. Treatment was begun 8 days after tumor graft.

increased compared to that in the oil-treated or non-treated tumor-bearing controls. This count varied from one rat to another as in the experiment reported in table 1. In the group of rats treated with five doses of CNCC, we counted 22, 60, 64, 80, 88, 126, 150, and 186 nodules at the surface of the lungs. The individual counts of lung nodules in the control group were 0, 0, 0, 4, 6, 7, 7, and 20. The degree of lymph node invasion appeared to be decreased in all sites, compared to the controls.

The effect of the number of CNCC doses in protocol treatment was tested. Table 1 shows that the rats treated with a single dose of 90 mg/kg presented a high count of metastases in the lungs (four rats with > 80 nodules). The tumor size did not differ from that in the paraffin oil-treated rats, while the loss of weight exceeded 30%. The percentage of rats with lymph node metastases was low, except at the mediastinal site. The treatment was prolonged to three 50-mg/kg doses. Loss of weight was observed, but the tumor growth rate appeared to be significantly modified. The degree of metastasis was decreased. The number of rats with a high count of metastatic nodules in the lungs was elevated.

Two hydrosoluble nitrosoureas were used and their effects were compared to those of CNCC. CZT and HECNU were injected ip. An identical loss of body weight was observed (table 2), while survival time and tumor growth were little affected in both cases. The count of lung metastases was considerably increased. Invasion of lymph node areas was little modified. Occasionally, CZT was given to tumor-bearing rats at smaller doses, 20% and 10% of the usual 10-mg/kg dose, repeated weekly for 5 weeks. We did not observe loss of body weight, slow tumor growth rate, or increase of lung nodule count. The usual dose was followed by a mean count of 100 ± 12 lung nodules versus 10 ± 3 after 20% of CZT dose and 2 ± 4 after 10% of CZT dose. Two different chemotherapeutic drugs, CY and MTX, were used to treat rhabdomyosarcoma-bearing rats. We observed the antitumor effect of CY: a slowing of tumor growth rate (tumor diameter at Day 60: 20.6 mm in treated animals vs 4 mm in controls) and a delay of metastatic dissemination (1 vs 14 lung nodules). MTX was inactive on both the local tumor (39.8 vs 40 mm) and the metastatic spread (16 vs 14 nodules). No increase of lung nodules was noted.

Metastatic Potential of Tumors Obtained After Graft of CZT-Treated Lung Metastatic Tissue

Lung metastatic tissue originating from CZT-treated rats was grafted to syngeneic rats. Tumors grew at the site of graft and metastasized to the nodes and lungs in the absence of any treatment. These experiments were performed to determine if lung metastatic tissue had acquired a new pulmonary metastatic phenotype in response to the nitrosourea treatment.

Results show no significant differences between the mean number and distribution of lung nodules in the group of rats that received H_2O-treated lung tissue (control) compared to rats that received CZT-treated lung tissue.

Antitumor Effect of Nitrosourea in the Treatment of Other Tumors

The 13762 adenocarcinoma was usually metastatic to the lymph node sites and to the lungs. The survival time was generally short and the deaths of tumor-bearing rats were linked to tumor development accompanied by a dramatic loss of weight and a toxic state. Early treatment 8 days after the graft had been shown to cure rats in 100% of cases. The treatment of rats was initiated when the tumor reached 15 mm in diameter (15-day evolution time). The protocol was the same as previously described; rats received three weekly doses of CZT. Comparing the CZT-treated group to the H_2O-treated group, we observed a slowing of the tumor growth rate, as shown in figure 2, and an increase in weight loss. The survival time was prolonged. Dissemination to lymph nodes sites was less frequent, but we did see an invasion of mediastinal nonpulmonary tissue and a significant increase in the mean count of lung metastatic nodules (from 14 to 71) (table 3).

The Mc B6-1 fibrosarcoma of C57BL/6 mice was also treated by CZT at a dose of 20 mg/kg of body weight. We observed that the tumor growth rate was hardly modified by the treatment; macroscopic and microscopic examination of the lungs did not reveal any invasion by tumor.

Soft-tissue tumors induced by nickel in WAG rats were treated by CZT from the time of detection, according to the usual protocol. Five of nine rats treated with CZT showed lung dissemination with a high nodule count (> 100). Three of 11 rats treated with water as controls showed a pulmonary spread of tumor; only two had a high nodule count.

Tumor Behavior and Metastatic Dissemination in CNCC-Pretreated Rats

These experiments were performed to determine if the increase of metastases in the lung was related to a direct effect of the nitrosourea on the host. As shown in table 4, the mean survival time was shortened in the group of CNCC-pretreated rats. All of the rats of both groups were killed because of deterioration of health and enlargement of the tumor. The CNCC-pretreated rats had lost some weight and did not regain their normal weight for the 60 days of tumor evolution, despite the discontinuation of treatment. Metastatic dissemination to the lymph node areas was not greatly modified by the pretreatment. Metastatic invasion of lungs was slightly more frequent in the CNCC-pretreatment group and the

TABLE 1.—Effect of CNCC treatments on tumor growth and dissemination, compared to paraffin oil treatment controls

Treatment of rats	Survival time* (days)	Tumor size (mm)	Weight of rats (g)	No. of rats with metastases/total No. in group					No. of lung nodules	P value†
				Nodal sites				Pulmonary site		
				Inguinal	Axillary	Mediastinal	Lumbaraortic			
Paraffin oil	74 ± 1	36 ± 1	190 ± 7	5/5	2/5	4/5	0/5	4/5	10 ± 5	< 0.02
CNCC (1 dose of 90 mg/kg)	60 ± 2	36 ± 3	166 ± 11	0/10	0/10	9/10	0/10	10/10	102 ± 31	
Paraffin oil (3 doses)	57 ± 2	36 ± 2	184 ± 4	10/10	1/10	3/10	5/10	6/10	2 ± 1	< 0.01
CNCC (3 doses of 50 mg/kg)	54 ± 2	24 ± 2	127 ± 4	3/10	0/10	3/10	1/10	9/10	73 ± 25	
Paraffin oil (5 doses)	66 ± 2	30 ± 2	198 ± 3	5/8	3/8	4/8	3/8	5/8	8 ± 3	< 0.01
CNCC (5 doses of 50 mg/kg)	66 ± 2	28 ± 2	139 ± 6	1/8	0/8	0/8	1/8	8/8	98 ± 19	

*Survival time measurements for CNCC-treated group only. Controls were killed at a similar period.
†Statistical significance of the differences calculated by Wilcoxon test.

TABLE 2.—Effect of CZT and HECNU on tumor growth and metastatic dissemination of a transplanted rat rhabdomyosarcoma

Treatment of rats	Survival time* (days)	Tumor size† (mm)	Weight of rats (g)	No. of rats with nodal metastases/ total No. in group				No. of lung nodules	P value
				Inguinal	Axillary	Mediastinal	Lumbaraortic		
H_2O	73 ± 2	34 ± 1	203 ± 5	6/9	4/9	0/9	0/9	2 ± 1	< 0.01
CZT	73 ± 2	36 ± 1.5	158 ± 6	4/9	6/9	5/9	3/9	100 ± 12	< 0.05
HECNU	73 ± 5	31 ± 2	163 ± 5	6/10	4/10	2/10	0/10	155 ± 31	

*Mean ± SE.
†Mean diameter ± SE.

TABLE 3.—Effect of CZT treatment on tumor growth and metastatic dissemination of 13762 adenocarcinoma in Fischer rats

Treatment of rats	Survival time (days)	Weight of rats (g)	Tumor size (mm)	No. of rats with metastases/ group of 10 rats					No. of lung nodules
				Nodal sites				Pulmonary site	
				Axillary ipsilateral	Axillary contralateral	Mesenteric	Mediastinal		
H_2O	31 ± 0.8	154 ± 5.5	42 ± 1.8	9	3	9	0	10	15 ± 3.3
CZT	34 ± 0.3	132 ± 4.2	25 ± 1.1	8	1	5	3	10	71 ± 20.4
Statistical significance	0.001*	0.01*	0.001*						< 0.05†

*Calculated by Student's t test.
†Calculated by Wilcoxon test.

TABLE 4.—Effect of CNCC pretreatment of rats on the metastatic dissemination of 9-4/0 rhabdomyosarcoma

Pretreatment of rats	Survival time (days)	Weight of rats (g)	No. of rats with metastases/total No. in group					No. of lung nodules	P value*
			Nodal sites				Pulmonary site		
			Inguinal	Axillary	Mediastinal	Lumbaraortic			
None	75 ± 3	320 ± 8	4/10	10/10	10/10	10/10	9/10	14 ± 5	< 0.02
Paraffin oil	69 ± 0	360 ± 3	7/10	2/10	4/10	1/10	5/10	1 ± 1	< 0.01
CNCC + paraffin oil	62 ± 6	291 ± 18	4/9	1/9	6/9	0/9	9/9	22 ± 5	< 0.05

*Calculated by Wilcoxon test.

number of metastases was more elevated than in untreated controls (fig 3). It can be noted that in this experiment, the paraffin oil-pretreated rats had fewer lung metastases than the untreated rats.

Postoperative Treatment with CNCC

To determine if the CNCC could have a direct effect on tumor cells established in the lungs, we treated rats after surgical excision of the rhabdomyosarcoma.

In the oil-treatment group of ten rats, there was no tumor recurrence among four rats. Among the six rats with recurrence, three exhibited metastases at the lumbaraortic site and two had a small number of lung metastases. In the CNCC-treated group, the frequency of lung dissemination was elevated (eight of ten rats) independently of the local recurrence. The lung nodule count was greatly increased in the group of rats with tumor recurrence (from nine to 50) (table 5).

Histology

Pulmonary metastatic lesions were of two types: *type I*—small, from one to three per lung section, preferentially localized under pleura; and *type II*—large or mixed, numerous (up to 31/lung section, sometimes confluent), localized under pleura and within parenchyma (in the proportion of 1 to 2). Metastatic tissue was well-differentiated and was similar to the primitive tumor. Pulmonary metastases present in rats that received paraffin oil, H_2O, or nothing were type I.

Pulmonary metastases in rats that received nitrosoureas were generally type II, occasionally type I. The total number of metastases counted in five sections, each corresponding to the five lung lobes, paralleled the number of nodules macroscopically counted. In most rats, we observed focal emphysema associated with lesions described by Spencer (11) in the chronic pneumonitis of cholesterol-type: presence of diffuse interstitial, peribronchial, and perivascular granulomas composed of numerous plasma cells and a few lymphocytes. Moreover, in some fields, the alveoli were filled with macrophages. We never observed fibrinoid necrosis in the vascular walls. In any case, this lesion was not associated with the presence of metastasis or drug toxicity, since it was observed in every rat without metastases and in untreated animals. In nitrosourea-treated animals, we did not observe interstitial pulmonary fibrosis as reported by Lee et al (12).

DISCUSSION

We have ascertained the amplification of lung metastatic dissemination in two different rat tumor models as a consequence of nitrosourea treatment. Some authors have previously reported the increase of pulmonary me-

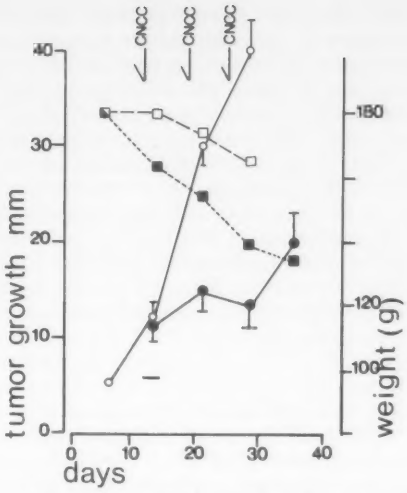

FIGURE 2.—Adenocarcinoma 13762-bearing rats. Tumor growth rate with CZT (●——●) or H_2O (○——○) treatment. Body weight with CZT (■——■) or H_2O (□——□) treatment.

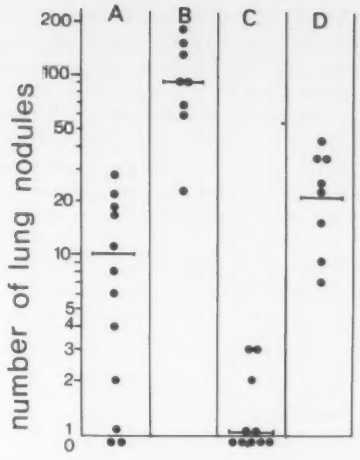

FIGURE 3.—Individual count of lung metastatic nodules in rats pretreated with weekly ingestions of oil (C) or CNCC in oil (D), and grafted 8 days later with tumor cells, compared with tumor-bearing rats treated with oil (A) or CNCC in oil (B).

tastases after chemotherapy or radiotherapy. Van Putten et al (5) reported a marked increase in lung metastases caused by cytostatic drugs, especially CY. A screening of drugs by treatment of mice prior to the iv injection of tumor cells could define their enhancing capacity on lung colony formation as shown by Carmel and Brown (13). For example, lomustine and semustine multiplied the formation of lung colonies by a factor of 4 and 6, respectively, whereas CY did so by a factor of 100 to > 1000. Steel and Adams (3) reported a similar increase in lung colonies after iv injection of Lewis lung carcinoma cells by previous treatment of mice with CY. Milas et al (4) confirmed these results with a murine fibrosarcoma model. Moore and Dixon (6) reported observations on the increased spread of metastasis of a transplantable rat mammary tumor by ^{60}Co irradiation in combination with CY. These findings compared well with the work of Dao and Yogo (14), who reported the enhancement of spontaneous metastases of rat mammary tumor by x-irradiation. More recently, Brown (15) showed that 2000 R of irradiation to the thorax increased the lung colonization of KHT sarcoma. Van Putten et al (5) confirmed these results with 1000 R of thoracic x-irradiation and 500 R of total-body x-irradiation.

Most of the work on lung colony formation has been done using the iv injection of tumor cells, an assay described by Hill and Bush (16). This assay makes possible the study of the fixation and growth of tumor cells in the lung rather than the release of cells from the primary tumor; the above experiments have demonstrated that drugs could enhance the fixation of tumor cells in the lung.

It is difficult to compare artificial lung colony formation with metastatic dissemination of an established tumor, as emphasized by the work of Stackpole (17). In many respects, these highly artificial models are not comparable to the clinical situation in human cancer. The panel of multitransplanted tumors used for the screening of antitumor drugs includes the Lewis lung carcinoma, a tumor considered representative of solid tumors with me-

TABLE 5.—Effects of postsurgical CNCC treatment on the development of metastases

Treatment of rats	Survival time (days)	Postoperative local recurrences	No. of rats with metastases/ total No. in group: Lumbaraortic nodes	No. of rats with metastases/ total No. in group: Lungs	No. of lung nodules	*P* value*
Surgical ablation followed by paraffin oil treatment	93	6 rats with recurrence	3/6	2/6	3 ± 1	< 0.05
		4 rats without recurrence	0	0	0	
Surgical ablation followed by CNCC treatment†	83	4 rats with recurrence	1/4	4/4	50 ± 19	< 0.05
		6 rats without recurrence	2/6	4/6	9 ± 3	

*Calculated by Wilcoxon test.
†3 weekly doses of 50 mg/kg of body weight orally.

tastasizing properties. It is therefore surprising that the capacity of anticancer drugs to enhance metastasis to the lung has not yet been reported, although many drugs used in human cancer have been previously studied in the Lewis lung carcinoma: eg, investigations by Sacchi et al (18), Greco et al (19), Martini et al (20), and Ovejera et al (21). This could mean either that the phenomenon is not specifically sought or that this model of a rapidly growing tumor is not adapted to detect such a specific effect. The work of Moore and Dixon (6) and our studies better approximate the therapeutic situation. Indeed, in these models, there is a prolonged phase of local tumor growth with a secondary metastatic dissemination phase by the lymphatic route to superficial and deep nodes and by the blood pathway to the lungs.

In our study, 45 of 46 nitrosourea-treated rats (99%) had lung metastases with a 95 ± 9.7 nodule count at the surface of the lungs. In the control group, 29 of 41 rats (71%) had lung metastases with a 7 ± 1.5 mean nodule count.

Histology confirmed the rhabdomyosarcoma origin of the metastases and the validity of the visual count technique. Lung metastatic amplification was observed after five weekly doses of CNCC, whereas a single administration of a high dose was already sufficient to provoke the phenomenon in four of ten rats. Small doses of nitrosoureas did not provoke metastatic amplification.

This amplifying effect was not limited to one tumor model. A similar effect was observed after treatment of Fischer rats bearing 13762 adenocarcinoma and of WAG rats bearing Ni-induced tumor.

Metastatic amplification did not depend on the route of administration (orally or ip) or on the drug vehicle (oil or water). This amplifying effect was rediscovered with two other nitrosoureas, CZT and HECNU. Two other drugs, CY and MTX, did not show a similar effect. CY had an antitumor effect; it induced a slowing of growth rate of the primary tumor and delayed the dissemination of metastases without amplifying effect. MTX was inactive on both the local tumor and metastatic spread.

There were several possible explanations of this amplifying effect. The most evident explanation was that immunosuppressive activity of these drugs enhanced the metastatic spread. For example, Fidler et al (22) have shown that fibrosarcoma formed more pulmonary tumor colonies in immunosuppressed than in normal mice. Hayakawa et al (23) established a correlation between the antitumor immunity following cryosurgery of the primary tumor and the enhancement of metastatic spread. Sesharadi et al (24) have shown that mice immunosuppressed by hydrocortisone were more susceptible after x-irradiation to the metastatic dissemination of fibrosarcoma. The destruction of cells responsible for natural cell-mediated cytotoxicity which controls the dissemination of tumor cells in the blood, as shown by Hanna et al (25), or in the lung tissue, as shown by Nolibé et al (26), by cytotoxic drugs, as reported by Mantovani et al (27), could explain an increased number of surviving tumor cells capable of forming lung metastases. Moreover, preliminary experiments on the pulmonary clearance of ^{125}I-labeled tumor cells injected iv, as used by Fidler et al (28) to study their colonization potential in nitrosourea-pretreated rats compared to controls, showed a decreased capacity to kill tumor cells.

The second hypothesis was to explain amplifying effect by a direct effect on the host, especially on the lung tissue, as observed after thoracic x-irradiation. To test this hypothesis we used rats pretreated with five injections of CNCC before graft of the tumor, to produce an enhancement of lung metastatic spread.

Our results did not clearly confirm this hypothesis. The CNCC-pretreated rats had only a slight increase in lung nodule count. The results of postoperative treatment with CNCC showed that the amplifying effect on metastases was preferentially associated with recurrences of local tumor. Two hypotheses could explain this fact.

(*a*) Nitrosoureas could cause an excessive fragility of the vessel walls and increase the number of cells leaving the primary tumor. Vlodavsky et al (29) had shown that metastatic cells were able to degrade the basal lamina of subendothelium and thereby allowed their extravasation.

(*b*) There could be in vivo modification of cellular subpopulations of the tumor in response to treatment. The heterogeneity of tumors is currently the subject of intensive research in several laboratories, namely by Fidler et al (30), Poste et al (31), and Lohmann-Matthes et al (32). Most tumors are heterogeneous by multiple characters including susceptibility to drugs, as shown by Tsuruo and Fidler (33) and Heppner et al (34). Previous work in our laboratory has determined that the rhabdomyosarcoma 9-4 was heterogeneous by several characters (7,35). Cloning had permitted the isolation of high and low metastatic clones. The parental cell line was shown to have a low metastatic potential. This fact, ie, the controlled expression of highly metastatic cells by the microenvironment inside a same tumor, is in good agreement with the work of Poste et al (36). They showed that metastatic properties of clones isolated from the B16 melanoma are extensively heterogeneous and differ widely in their metastatic abilities. They have observed changes of metastatic potential of heterogeneous tumor cell populations following the killing of subpopulations by means of their particular susceptibility to drugs. New subpopulations could emerge containing phenotypically diverse subpopulations. The new population obtained could express a new and unforeseeable metastatic potential. Thus, the nitrosoureas could act by destruction of stabilizing clones or by inducing mutagenesis leading to the appearance of new clones and the acquisition of a new highly metastatic tumor cell population. Nitrosoureas are known to be highly mutagenic and a direct relation between genetic mutation capacity and invasiveness of tumor cells has been reported by Cifone

and Fidler (37). We could not demonstrate a new selected metastasizing population by transplantation of metastatic nodules originating from lungs of nitrosourea-treated rats. This experiment is not definitive, however, because the supposedly new phenotype could be drug-dependent or unstable.

At this time, no definitive conclusion will be advanced to explain enhancement of lung metastatic dissemination since further studies are necessary.

The question of the complex biological effect of antitumor drugs on facilitation of tumor progression was analyzed by Kerbel and Davies (38), who warned against the use of anticancer drugs that are strongly mutagenic. We believe that our present results justify including a specific study of possible adverse effects of all antitumor drugs on metastatic dissemination.

Adjuvant chemotherapy is being used more and more often in the treatment of human cancers, and drugs with a risk of amplifying metastatic dissemination should not be administered in such cases.

REFERENCES

1. GOLDIN A. Historical overview of nitrosourea development. *In* Nitrosoureas in Cancer Treatment (Serrou B, Schein PS, and Imbach JL, eds). Amsterdam, Elsevier/North-Holland Biomedical Press, 1981, pp 3-12.
2. IMBACH JL, MARTINEZ J, OIRY J, ET AL. New nitrosourea derivatives and related compounds. *In* Nitrosoureas in Cancer Treatment (Serrou B, Schein PS, and Imbach JL, eds). Amsterdam, Elsevier/North Holland Biomedical Press, 1981, pp 123-138.
3. STEEL GG, and ADAMS K. Enhancement by cytotoxic agents of artificial pulmonary metastasis. Br J Cancer 36:653-658, 1977.
4. MILAS L, MALENICA B, and ALLEGRETTI N. Enhancement of artificial lung metastases in mice caused by cyclophosphamide. Cancer Immunol Immunother 6:191-196, 1979.
5. VAN PUTTEN LM, KRAM LKJ, VAN DIERENDONCK HHC, ET AL. Enhancement by drugs of metastatic lung nodule formation after intravenous tumor cell injection. Int J Cancer 15:588-595, 1975.
6. MOORE JV, and DIXON B. Metastasis of a transplantable mammary tumor in rats treated with cyclophosphamide and/or irradiation. Br J Cancer 36:221-226, 1977.
7. SWEENEY FL, POT-DEPRUN J, POUPON MF, ET AL. Heterogeneity of the growth and metastatic behavior of cloned cell lines derived from a primary rhabdomyosarcoma. Cancer Res 42:3776-3782, 1982.
8. SEGALOFF A. Hormones in breast cancer. Recent Prog Horm Res 22:351-374, 1966.
9. POUPON MF, LESPINATS G, KOLB JP, ET AL. Immunity to a 3-methylcholanthrene-induced fibrosarcoma in the C57BL/6 mouse: in vivo analysis by the adoptive tumor neutralization test. JNCI 62:989-994, 1979.
10. SIEGEL S. *In* Nonparametric Statistics for the Behavioral Sciences. New York, McGraw-Hill, 1956, pp 116-127.
11. SPENCER H. Pathology of the Lung, 3rd ed. Pergamon Press, 1977, vol I.
12. LEE W, MOORE RP, and WAMPLER GL. Interstitial pulmonary fibrosis as a complication of prolonged methyl-CCNU therapy. Cancer Treat Rep 62:1355-1358, 1978.
13. CARMEL RJ, and BROWN JM. The effect of cyclophosphamide and other drugs on the incidence of pulmonary metastases in mice. Cancer Res 37:145-151, 1977.
14. DAO TL, and YOGO H. Enhancement of pulmonary metastases by x-irradiation in rats bearing mammary cancer. Cancer 20:2020-2025, 1967.
15. BROWN JM. The effect of lung irradiation on the incidence of pulmonary metastases in mice. Br J Radiol 46:613-618, 1973.
16. HILL RT, and BUSH RS. A lung colony assay to determine the radiosensitivity of the cells of a solid tumor. Int J Radiat Biol 15:435-444, 1969.
17. STACKPOLE GW. Distinct lung-colonizing and lung metastasizing cell populations in B16 mouse melanoma. Nature 289:798-800, 1981.
18. SACCHI A, CALABRESI F, GRECO C, ET AL. Different metastatic potential of in vitro and in vivo lines selected from Lewis lung carcinoma: correlation with response to different bleomycin schedulings. *In* Invasion Metastasis. Basel, Karger, 1981, vol 1, pp 227-238.
19. GRECO C, CALABRESI F, CAPUTO M, ET AL. Adriamycin effect on the Lewis lung carcinoma: comparison between the original line and its derivative subline. Eur J Cancer 16:1251-1255, 1980.
20. MARTINI A, DONELLI MG, MANTOVANI A, ET AL. Antineoplastic activity and pharmacokinetics of adriamycin and daunomycin in tumor bearing mice. Oncology 34:173-178, 1977.
21. OVEJERA AA, JOHNSON RK, and GOLDIN A. Growth characteristics and chemotherapeutic response of intravenously implanted Lewis lung carcinoma. Cancer Chemother Rep Part 2, vol 5 (1):111, 1975.
22. FIDLER IJ, GERSTEN DM, and KRIPKE ML. Influence of immune status on the metastasis of three murine fibrosarcomas of different immunogenicities. Cancer Res 39:3816-3821, 1979.
23. HAYAKAWA K, YAMASHITA T, SUZUKI K, ET AL. Comparative immunological studies in rats following cryosurgery and surgical excision of 3-methylcholanthrene-induced primary autochthonous tumors. Gan 73:462-469, 1982.
24. SESHARADI M, PODUVAL TB, and SUNDARAM K. Studies on metastases. I. Role of sensitization and immunosuppression. JNCI 63:1205-1207, 1979.
25. HANNA N, and FIDLER IJ. Role of natural killer cells in the destructive of circulation tumor emboli. JNCI 65:801-809, 1980.
26. NOLIBE D, BEREL E, MASSE R, ET AL. Characterization of a major natural killer activity in rat lungs. Biomedicine 35:230-234, 1981.
27. MANTOVANI A, LUINI W, PERI G, ET AL. Effect of chemotherapeutic agents on natural cell-mediated cytotoxicity in mice. JNCI 61:1255-1261, 1978.
28. FIDLER IJ, GERSTEN DM, and RIGGS C. Relationship of host immune status to tumor cell arrest, distribution and survival in experimental metastasis. Cancer 40:46-55, 1977.
29. VLODAVSKY I, ARIAV Y, ATZMON R, ET AL. Tumor cell attachment to the vascular endothelium and subsequent degradation on the subendothelial extracellular matrix. Exp Cell Res 140:149-159, 1982.
30. FIDLER IJ, GRUYS E, CIFONE MA, ET AL. Demonstration of multiple phenotype diversity in a murine melanoma of recent origin. JNCI 67:947-949, 1981.
31. POSTE G, DOLL J, and FIDLER IJ. Interactions among clonal subpopulations affect stability of the metastatic phenotype in polyclonal populations of B16 melanoma cells. Proc Natl Acad Sci USA 78:6226-6230, 1981.
32. LOHMANN-MATTHES ML, SCHLEICH A, SHANTZ G, ET AL. Tumor metastases and cell-mediated immunity in a model system in DBA/2 mice. VII. Interaction of metastasizing and nonmetastasizing tumors with normal tissue in vitro. JNCI 64:1413-1414, 1980.
33. TSURUO T, and FIDLER IJ. Differences in drug sensitivity among tumor cells from parental tumors, selected variants and spontaneous metastases. Cancer Res 41:3058-3064, 1981.
34. HEPPNER GH, DEXTER DL, DENUCCI T, ET AL. Heterogeneity in drug sensitivity among tumor cell subpopulations of a single mammary tumor. Cancer Res 38:3758-3763, 1978.
35. POUPON MF, JUDDE JG, POT-DEPRUN J, ET AL. Variable susceptibility to NK activity of cloned cell lines derived from a primary rat rhabdomyosarcoma: relationship to metastatic potential. Br J Cancer 48:75-82, 1983.
36. POSTE G, DOLL J, BROWN AE, ET AL. Comparison of the metastatic properties of B16 melanoma clones isolated from cultured cell lines, subcutaneous tumors and individual lung metastases. Cancer Res 42:2770-2778, 1982.
37. CIFONE MA, and FIDLER IJ. Increasing metastatic potential is asso-

ciated with increasing genetic instability of clones isolated from murine neoplasms. Proc Natl Acad Sci USA 78:6949–6952, 1981.

38. KERBEL RS, and DAVIES AJS. Facilitation of tumor progression by cancer therapy. Lancet 977–978, Oct 30, 1982.

Differences in Effects of Immediate and Delayed Hyperoxia Exposure on Bleomycin-Induced Pulmonary Injury[1,2]

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Several reports have suggested that patients treated with bleomycin may be at greater risk of developing respiratory failure when exposed to elevated concentrations of oxygen. We studied the interactions of bleomycin and hyperoxia in Syrian golden hamsters. Animals were instilled intratracheally with bleomycin at a dose of 0.5 unit/100 g of body weight, followed immediately by exposure to 70% oxygen for 72 hours. Mortality was 90% in these hamsters, compared to 15% in an age-matched control group treated with bleomycin alone. Postmortem studies revealed that pathologic changes were confined to the lungs which showed severe, hemorrhagic, diffuse alveolar damage. To determine the effect of delaying exposure to hyperoxia, bleomycin at a dose of 0.5 unit/100 g of body weight was instilled and animals were kept in room air for 1 and 2 months before exposure to 70% or 100% oxygen for 72 hours. No significant increase in mortality or interstitial pneumonitis and fibrosis was seen in these groups during or after the hyperoxic exposures. Mortality in controls treated with saline and hyperoxia was zero. We conclude that simultaneous treatment with bleomycin and hyperoxia results in a synergistic effect on mortality and on the development of pulmonary fibrosis. However, there is no synergism if the hyperoxic exposure is delayed for at least 1 month following bleomycin treatment. [Cancer Treat Rep 68:759–764, 1984]

Bleomycin is a highly effective antineoplastic agent, but its usefulness is limited by significant pulmonary toxic effects. Respiratory abnormalities occur in 11% of patients with a 1%–2% mortality, if the cumulative dose is < 450 units (1). The risk of morbidity and mortality increases dramatically at doses > 450 units. Other risk factors for bleomycin toxicity are increasing age and administration with other chemotherapeutic agents (1–3). Conflicting clinical studies (4,5) suggest that patients who received bleomycin may have an increased susceptibility to pulmonary oxygen toxicity.

Lung injury induced by bleomycin has been extensively studied in laboratory animals (6–9). Increased mortality and pulmonary injury were found when bleomycin was followed immediately by hyperoxia (10–12) and also when hyperoxia exposure occurred during the acute injury phase (13). The effects of hyperoxia delivered after bleomycin-induced pulmonary disease has developed had not been assessed. The purpose of this study is to compare the effects of hyperoxia in the bleomycin-injured lung when the hyperoxia is delivered immediately after bleomycin to the effects seen when it is given later.

METHODS

Animals.—Male Syrian golden hamsters (LVG:LAK, Charles River Lab, Inc, Lakeview, NJ) weighing 110–130 g were used. They were housed in individual cages and given Purina rat chow and water ad libitum. The animals were randomly assigned at the time of hyperoxia exposure to treatment groups summarized in table 1.

Bleomycin treatment and exposure to hyperoxia.—This protocol has been previously described in detail (10). Briefly, a single transoral intratracheal instillation of 0.5 unit of bleomycin sulfate (Blenoxane, donated by Bristol Labs, Syracuse, NY) in 0.33 ml of saline/100 g of body weight was administered. Control hamsters received the same volume of saline. Hamsters were exposed to hyper-

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TABLE 1.—Treatment groups*

Group No.	No. of hamsters†	Oxygen level (%)‡	Days between bleomycin treatment and exposure
1	20	70	0
2	20	20	0
3	9	70	30
4	9	100	30
5	9	20	30
6	9	70	60
7	9	20	60
8	9	100	60
9	9	20	60

* All bleomycin-treated hamsters received 0.5 unit of bleomycin/100 g of body weight.
†5 additional control hamsters were saline-instilled with each group.
‡60-day postbleomycin instillation exposures to 70% and 100% oxygen were performed at different times and are compared to their respective age and treatment-matched controls.

oxia for 72 hours. For the 70% exposure, the average oxygen concentration ranged from 70.4% to 71.2%. For the 100% oxygen exposure, the average oxygen concentration ranged from 96.2% to 97.3%. Average chamber temperature ranged from 23°C to 26°C and relative humidity from 60% to 78%. During the exposure period, the hamsters were in their individual cages and were given food and water ad libitum. Another group of 20 hamsters were treated with a single intratracheal instillation of 0.25 ml of 4% thioglycollate (14). This was used as a control to determine whether nonspecific injury and hyperoxia produced similar results to bleomycin and hyperoxia.

Mortality.—The animals were observed daily for 30 days after the hyperoxia exposure. Animals dying spontaneously had complete necropsies performed within 12 hours of death.

Histopathology and morphometry.—The remaining hamsters were killed 30 days after the hyperoxia exposure with a lethal dose (60 mg/kg of body weight) of ip pentobarbital sodium (Nembutal Sodium, Abbott Labs, N Chicago, IL). The lungs were inflated in situ with 2.5% gluteraldehyde in phosphate buffer (pH 7.4) at a transpulmonary pressure of 20 cm of water. The volume of glutaraldehyde used for fixation was recorded and used as an estimate of lung volume, as described by Hayatdavoudi et al (15). Two parasagittal sections from each lung, which included most of the pulmonary parenchyma, were processed for paraffin embedding. Sections were cut at 6 μm, stained with hematoxylin and eosin, and studied by light microscopy. The percent of the lung which was diseased in animals exposed to hyperoxia at 30 days after bleomycin treatment was determined morphometrically according to methods previously described (10,16). A total of 104 slices were quantitated. The average number of points evaluated was 1140/animal.

RESULTS

Bleomycin and Immediate Hyperoxia Exposure

Mortality data are shown in table 2. Hamsters treated with concomitant bleomycin and hyperoxia (Group 1) died, with a profound mean weight loss of 30 g and with lung disease. Hamsters that died ⩽ 7 days after bleomycin instillation (n = 7) had vascular congestion, perivascular edema, intra-alveolar hemorrhage, fibrin, and edema, with a modest increase in segmented leukocytes, macrophages, and lymphocytes. All animals dying after 7 days (n = 11) showed proliferative diffuse alveolar damage. This was manifested by interstitial edema, increased interstitial macrophages, lymphocytes, and segmented leukocytes, including eosinophils and proliferation of fibroblasts. Varying degrees of Type II pneumocyte hyper-

TABLE 2.—Thirty-day postexposure mortality in bleomycin-treated hamsters

	Interval between 0.5 unit of bleomycin/100 g of body weight and hyperoxia		
	0 days (%)	30 days (%)	60 days (%)
70% oxygen for 72 hrs	90	11	0
100% oxygen for 72 hrs	Not done	11	0
20% oxygen for duration of experiment	15	11	0

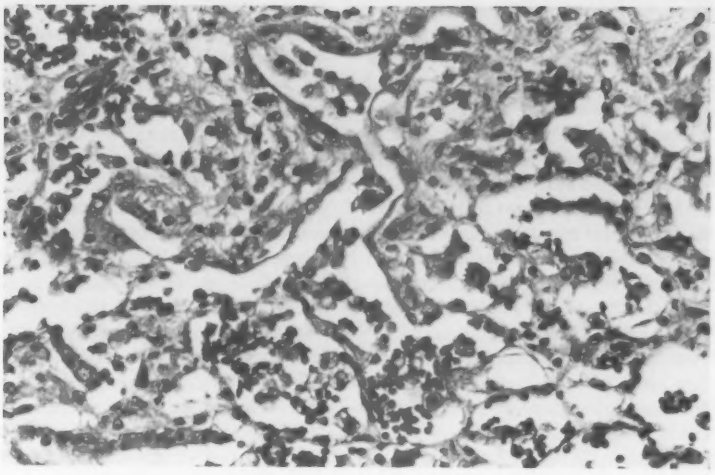

FIGURE 1.—Lung from hamster (Group 1) that died 11 days after treatment. Proliferative phase of diffuse alveolar damage is present, with thickened interstitium with increased inflammatory cells, Type II pneumocyte hyperplasia, and intra-alveolar cellular debris and hyaline membranes.

plasia could be seen, which became more prominent the longer the animal lived. Hyaline membranes, focal intra-alveolar hemorrhage, and organization of alveolar debris by fibroblasts were also present (fig 1). Histologic examination of the gastrointestinal tract, liver, heart, kidneys, and bone marrow showed no significant pathologic lesions.

Only two hamsters from Group 1 lived 30 days after treatment with bleomycin and hyperoxia. Both hamsters had severe interstitial pneumonitis with fibrosis when killed. In many regions, there was profound remodeling of the alveolar architecture, resembling honeycomb lung. This remodeled lung tended to be localized to the peribronchial region where some airway dilatation was also seen (fig 2).

Three hamsters from Group 2 died 13-25 days after

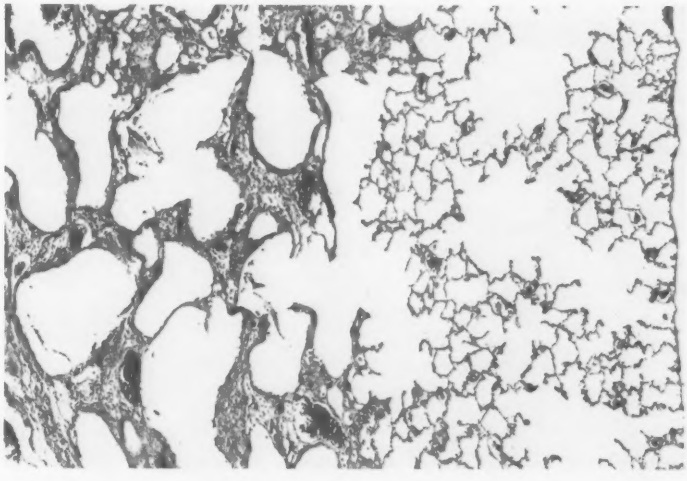

FIGURE 2.—Lung from hamster (Group 1) treated 30 days earlier with 0.5 unit of bleomycin, followed by exposure to 70% oxygen for 72 hrs. Pneumonitis with fibrosis is localized to the peribronchial regions, with dilatation of the larger airways. Peripheral parenchyma is spared.

bleomycin treatment alone. All had well-developed proliferative diffuse alveolar damage, but it was less severe than that seen in animals dying from bleomycin and hyperoxia. Survivors that were killed at 30 days also had less severe interstitial pneumonitis than that seen in the two survivors of bleomycin and hyperoxia treatment. The distribution of the interstitial disease also tended to be parabronchial, with occasional extension to the pleura, but airway dilatation was not a prominent feature. Additionally, seven (10% mortality) hamsters from Groups 3-9 died within the first 30 days prior to assignment to hyperoxia. These deaths were not included in the mortality data in table 2.

Bleomycin and Hyperoxia-Delayed Exposure

In Groups 3 and 4, hamsters were exposed to hyperoxia 30 days after bleomycin treatment. One hamster died in each of these groups and in the normoxia groups (table 2). The death in Group 4 occurred during the 100% oxygen exposure. Focal interstitial pneumonitis and fibrosis were present in the lung, but superimposed pulmonary edema and gram-positive rods were also present. It was not possible to determine whether the pulmonary edema preceded or followed the bacterial infection. The death of the hamster from Group 3 occurred 3 weeks after the 70% hyperoxia exposure; an animal from the bleomycin treatment-alone group (Group 5) also died 3 weeks after this exposure. Postmortem examinations were not done in these latter two deaths.

Weight gain and lung volumes were similar in the hyperoxia-exposed bleomycin-treated hamsters and in their matched bleomycin and room air controls. The histopathology of the lungs was similar in the bleomycin and hyperoxia (Groups 3, 4, 6, and 8) or bleomycin and room air (Groups 5, 7, and 9) hamsters when killed 30 days after exposure. There was interstitial pneumonitis with fibrosis, Type II pneumocyte hyperplasia, and regions of confluent disease with obliteration of alveolar architecture (fig 3).

To test whether differences more subtle than mortality or descriptive histology were present, the amount of lung disease was quantitated morphometrically. Groups 3-5 had 16.4% ± 11.2%, 22.6% ± 12.7%, and 28.6% ± 15.1% (mean ± SD) of the lung diseased, respectively. There was no significant difference between animals treated with bleomycin and then later exposed to 70% or 100% oxygen as compared to the room air bleomycin-treated hamsters.

The saline with hyperoxia or normoxia control hamsters had no spontaneous mortality during the study period and no significant lung disease detectable with histologic study. No animals treated with thioglycollate, alone or with hyperoxia, died spontaneously. Thioglycollate-treated hamsters killed 30 days after treatment showed focal epithelioid aggregates of macrophages. Simultaneous exposure to hyperoxia failed to alter this appearance.

DISCUSSION

Exposure to normobaric hyperoxia immediately after an intratracheal instillation of bleomycin results in potentiation of the pulmonary injury. This is manifested by

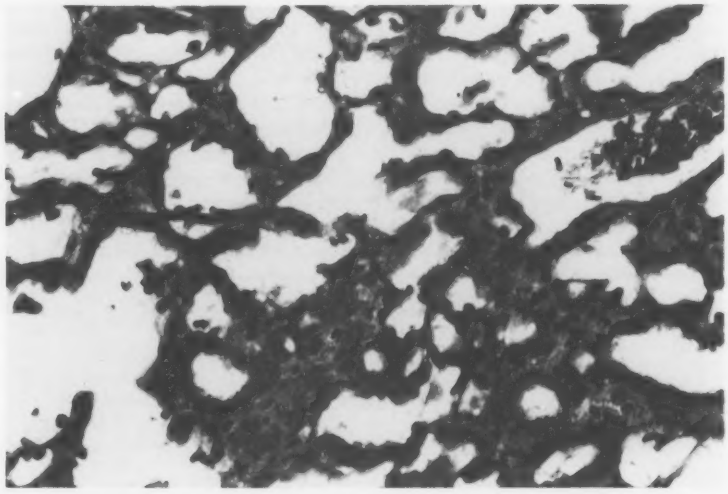

FIGURE 3.—Lung from hamster (Group 4) exposed to 100% oxygen 30 days after intratracheal instillation of 0.5 unit of bleomycin and killed 60 days after instillation. There is interstitial pneumonitis with interstitial thickening, inflammatory cells and collagen, and Type II pneumocyte hyperplasia.

an increase in mortality, an acceleration of the onset of death, and an increase in the amount of lung disease in survivors. This potentiation of the toxic effect of bleomycin treatment by simultaneous hyperoxia exposure has been observed experimentally in several rodent species (10–12,16). Toledo et al (12) reported decreased survival of mice treated with twice weekly sc bleomycin injections and continuous exposure to 40% oxygen, although they were unable to demonstrate that the lung was the site of this interaction. In the study reported here, we have documented the cause of death as lung disease, either as a diffuse alveolar hemorrhage in the early deaths or a severe proliferative diffuse alveolar damage in the later deaths. Additionally, histologic examination documents the normal appearance of the gastrointestinal tract, liver, kidney, heart, and bone marrow.

Hakkinen et al (11) treated mice with a single ip injection of bleomycin followed immediately by a 7-day exposure to 70% oxygen and found an increase in lung hydroxyproline content. However, they failed to find this increase when the hyperoxia was delayed until 9 days after the bleomycin injection. But Rinaldo and co-workers (13) report an enhancement of lung injury as measured by mortality and physiologic derangement by exposure to 60% oxygen, 8 and 21 days after treatment with intratracheally instilled bleomycin in hamsters. Both exposures to 60% oxygen were given during the prolonged inflammatory response seen in this model of bleomycin pulmonary injury.

In the study reported here, we also attempted to ascertain when the bleomycin-treated lung was at increased risk from oxygen toxicity; we focused on time points later than those previously studied. In this hamster model of bleomycin-induced lung injury, 30 days after instillation, the pneumonitis is healing, leaving residual fibrosis. Snider et al (7) have reported that there is some regression of the lesion by 60 days post bleomycin. Thus, the time points selected (30 and 60 days) were when the lung had largely recovered from the initial insult. When exposure to 70% oxygen had no effect, 100% oxygen was investigated. Untreated hamsters tolerate 100% oxygen for 3 days, but begin to die with a 4-day exposure. Thus, 72 hours of 100% oxygen was a substantial challenge. Yet, even this high dose of oxygen failed to significantly affect the mortality or pulmonary histopathology.

The pathogenic mechanisms of bleomycin injury and its potentiation by hyperoxia are not fully understood. It is likely that free radical injury is involved, although the cellular mediators of this injury may differ (16). Since potentiation is associated with the presence of a prominent acute inflammatory cellular component (13,16), the interaction of hyperoxia with inflammatory responses induced by other means is an important issue. Furthermore, when fibrosis and chronic inflammatory cells were the main tissue constituents, no potentiation was seen. We have found that intratracheally instilled thioglycollate rapidly elicits a threefold to fourfold increase in lavageable macrophages, which persists for 10 days, as well as an increase in segmented leukocytes within hours of instillation (14). This nonspecific injury then clears with minimal inflammatory residua and no fibrosis. This injury was not enhanced by exposure to 70% oxygen for 72 hours, thus showing that bleomycin produces a more specific injury which goes on to fibrosis and is enhanced by hyperoxia.

The effects of hyperoxia on bleomycin-exposed lung in clinical situations are controversial. Goldiner et al (4) described five young men treated for testicular carcinoma with bleomycin, who much later underwent a staging laparotomy. All five patients unexpectedly developed respiratory failure and died postoperatively. Two other similar cases have been reported as parts of large series (3,17). However, others have not been able to confirm these observations of increased mortality (5,18,19).

Frequently, life-threatening hypoxemia necessitates oxygen therapy, despite its possible toxicity. We conclude from our animal studies, as well as those of others, that simultaneous treatment with bleomycin and hyperoxia can act synergistically to enhance pulmonary injury. The increased susceptibility of the bleomycin-treated lung to oxygen toxicity may persist during the acute injury phase. But there is no synergism if the hyperoxic exposure is delayed until lung lesions have healed. In hamsters, 1 month seems to be adequate to terminate the acute phase when hyperoxia offers greater risks.

REFERENCES

1. CARTER SK, CROOKE ST, and UMEZAWA H. Bleomycin: Current Status and New Developments. New York, Academic Press, 1978.
2. WEISS RD, and MUGGIA FM. Cytotoxic drug induced pulmonary disease: update 1980. Am J Med 68:259–266, 1980.
3. BAUER KA, SKARIN AT, BALIKIAN JP, ET AL. Pulmonary complications associated with combination chemotherapy programs containing bleomycin. Am J Med 74:557–563, 1983.
4. GOLDINER PL, CARTON GG, CVITKOVIC E, ET AL. Factors influencing postoperative morbidity and mortality in patients treated with bleomycin. Br Med J 1:1664–1667, 1978.
5. DOUGLAS MJ, and COPPIN CML. Bleomycin and subsequent anesthesia: a retrospective study at Vancouver General Hospital. Can Anaesth Soc J 27:449–452, 1980.
6. ADAMSON IYR, and BOWDEN DH. The pathogenesis of bleomycin induced pulmonary fibrosis in mice. Am J Pathol 77:185–198, 1974.
7. SNIDER GL, HAYES JA, and KORTHY AL. Chronic interstitial pulmonary fibrosis in hamsters produced by endotracheal bleomycin. Am Rev Respir Dis 117:1099–1108, 1978.
8. RAISFELD IH. Pulmonary toxicity of bleomycin analogs. Toxicol Appl Pharmacol 56:326–336, 1980.
9. STARCHER BC, KUHN C, and OVERTON JE. Increased elastin and collagen content in the lungs of hamsters receiving an intratracheal injection of bleomycin. Am Rev Respir Dis 117:299–305, 1978.
10. TRYKA AF, SKORNIK WA, GODLESKI JJ, ET AL. Potentiation of bleomycin induced lung injury by exposure to 70% oxygen: morphologic assessment. Am Rev Respir Dis 126:1074–1079, 1982.
11. HAKKINEN PJ, WHITELEY JW, and WITSCHI HR. Hyperoxia, but not thoracic x-irradiation, potentiates bleomycin and cyclophosphamide-induced lung damage in mice. Am Rev Respir Dis 126:281–285, 1982.

12. TOLEDO CH, ROSS WE, HOOD CI, ET AL. Potentiation of bleomycin toxicity by oxygen. Cancer Treat Rep 66:359-362, 1982.
13. RINALDO JE, GOLDSTEIN RH, and SNIDER GL. Modification of oxygen toxicity after lung injury by bleomycin in hamsters. Am Rev Respir Dis 126:1030-1033, 1982.
14. GODLESKI JJ, MORTARA M, KATLER M, ET AL. Effect of thioglycollate on hamster lung macrophage antigen quantitated by flow cytometry. Am Rev Respir Dis 127:274, 1983.
15. HAYATDAVOUDI G, CRAPO JD, MILLER FJ, ET AL. Factors determining degree of inflation in intratracheally fixed rat lungs. J Appl Physiol 48:389-393, 1980.
16. TRYKA AF, GODLESKI JJ, SKORNIK WA, ET AL. Progressive pulmonary fibrosis in hamsters. Exp Lung Res 5:155-171, 1983.
17. DONOHUE JP, and ROWLAND RG. Complications of retroperitoneal lymph node dissection. J Urol 125:338-340, 1981.
18. MANDELBAUM I, WILLIAMS SD, and EINHORN LH. Aggressive surgical management of testicular carcinoma metastatic to lungs and mediastinum. Ann Thorac Surg 30:224-229, 1980.
19. PAGNONI A, RIBOLDI G, and VALAGUSSA P. Evaluation of lung ventilation and carbon monoxide-diffusing capacity in patients with nonseminomatous testicular tumors treated with bleomycin. Tumori 68:85-89, 1982.

Blood Levels of Alkylating Metabolites of Cyclophosphamide in the Mouse After Iv or Oral Administration[1,2]

Robert F. Struck[3,*] and David S. Alberts[4,5]

Blood levels of cyclophosphamide (CPA) and its alkylating metabolites in mice were determined after iv or oral administration of a 200-mg/kg dose of radiolabeled CPA. Individual metabolites (4-hydroxy-CPA, 4-keto-CPA, alcophosphamide, dechloroethyl-CPA, carboxyphosphamide, and phosphoramide mustard) were isolated from blood by solvent extraction and by thin-layer chromatography. Area-under-the-blood-decay-curve (AUC) values were determined for CPA and its alkylating metabolites to provide a basis for comparison of systemic exposure by iv or oral treatment. The total AUC values for alkylating metabolites of CPA was observed to be greater after iv administration, and AUC values for each of the individual metabolites were greater after iv treatment, except for the minor metabolite alcophosphamide. The methods in this study have been applied to one patient to date and will be used in future studies with other patients to determine relative AUC values for the oncolytic CPA metabolites 4-hydroxy-CPA and phosphoramide mustard. [Cancer Treat Rep 68:765–770, 1984]

A recent publication (1) reported that approximately 3.5 times the plasma alkylating activity was observed in patients with lymphoma when treated orally with cyclophosphamide (CPA) as compared to the same patients treated iv. To date, investigations on levels of CPA metabolites in the blood or plasma of experimental animals and humans have involved use of iv or ip administration (2–6). Juma et al (1) suggested that a different pattern of alkylating metabolites might be produced, depending on the use of an oral or iv route. Because of our ongoing interest in the metabolism of CPA, a study with mice was conducted to investigate this interesting possibility and also to compare blood levels of CPA and its alkylating metabolites following oral and iv administration.

The metabolism of CPA follows the pathway shown in figure 1. Metabolites of chief interest are 4-hydroxy-CPA, 4-keto-CPA, carboxyphosphamide, and phosphoramide mustard (PM). 4-Hydroxy-CPA is believed by most investigators of the mechanism of action of CPA to be the primary extracellular mediator of antitumor activity (5,7–9). Other investigators (6,10) believe that PM performs this role both extra- and intra-cellularly. A recent publication suggests an important role for imino-CPA (11). Carboxyphosphamide is the major excretion product (12,13).

The observations of Juma et al (1) are of significant clinical potential. If oral administration of CPA results in higher blood levels of the alkylating metabolites that mediate CPA's oncolytic effect, preferential use of this route should be considered in both clinical and experimental investigations.

MATERIALS AND METHODS

Cyclophosphamide.—CPA was obtained from Mead Johnson, Evansville, IN, dissolved in sterile water in the desired concentration, and ^{14}C-CPA (0.2 μCi/mouse) was added. ^{14}C-side-chain-labeled CPA was provided by Dr. Robert R. Engle, National Cancer Institute, Silver Spring, MD, and was purified by thin-layer chromatography to a purity of > 95%. Specific activity was 24 μCi/mg after purification.

Mice.—Six- to eight-week-old male DBA/2 mice (Jack-

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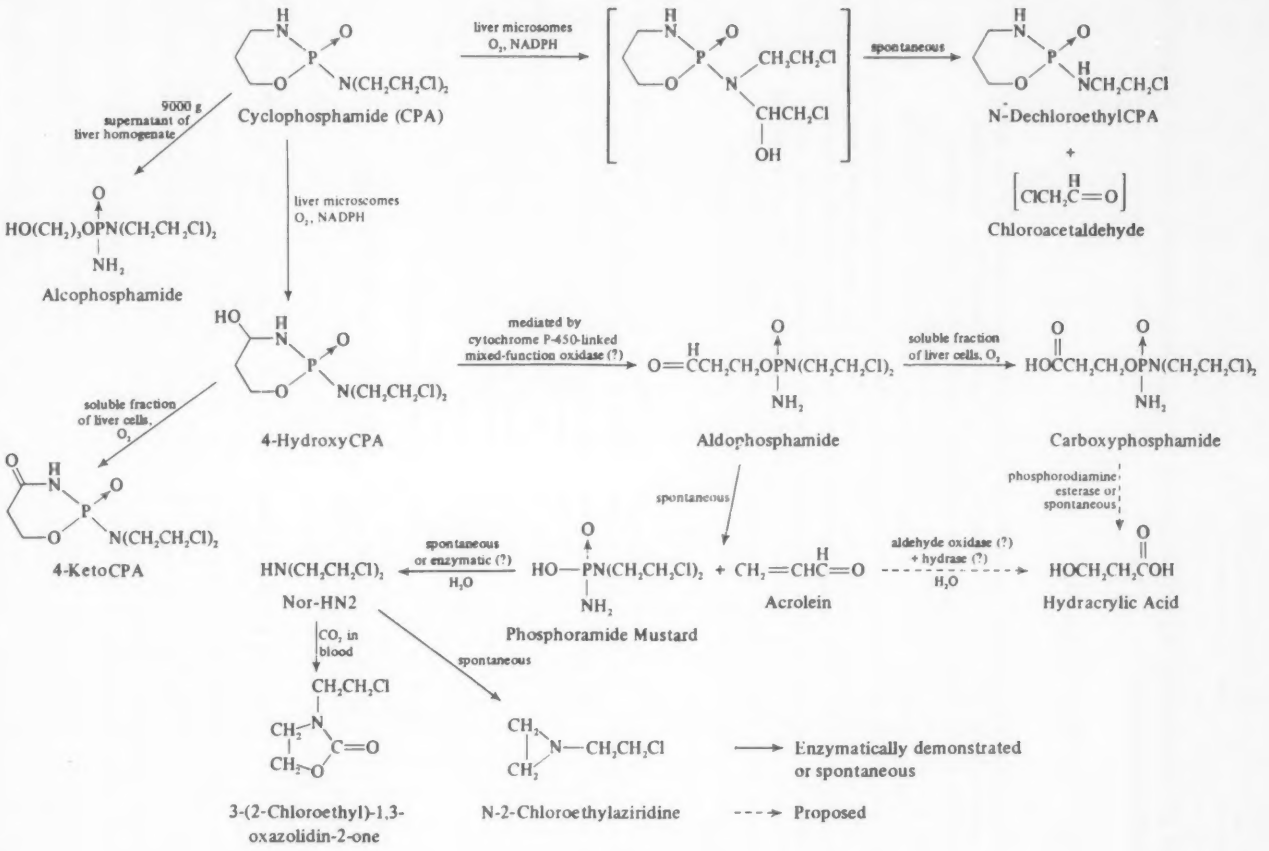

FIGURE 1.—Metabolism of CPA.

son Laboratory, Bar Harbor, ME) weighing approximately 20 g were used in these experiments.

Drug treatment and collection of blood.—Mice were treated in groups of five each with ^{14}C-CPA (200 mg/kg of body weight, 0.2 μCi/mouse) iv by tail vein or orally by direct instillation into the esophagus through a curved, blunt needle. At each sampling time after CPA administration (iv—5, 15, 30, 45, and 60 minutes; orally—15, 30, 60, and 90 minutes), groups of five mice were killed by decapitation and their blood was collected in iced tubes, each containing 0.25 ml of *N*-sodium bisulfite and 0.25 ml of *N*-sodium cyanide to trap 4-hydroxy-CPA as aldophosphamde cyanohydrin (9). Blood samples were shaken, immediately frozen, and stored at -20°C. A patient was treated with 1 g ^{14}C-CPA (200 μCi) by iv push for 5 minutes, and blood samples (3 ml) were collected at various times thereafter (8, 15, 38, 60, and 90 minutes; 2, 3, 4, 5, 6, 12, 18, and 24 hours) in tubes containing 0.5 ml each of *N*-sodium bisulfite and *N*-sodium cyanide. The blood samples were shaken, immediately frozen, and stored at -20°C. The same patient was treated 30 days later with 1 g of ^{14}C-CPA (200 μCi) by oral bolus. Blood samples (3 ml) were collected at various times thereafter (5, 15. 30, 60, and 90 minutes; 2, 3, 3.5, 4, 5, 6, 8, 13, and 24 hours) in tubes containing sodium bisulfite and sodium cyanide; the samples were shaken, frozen, and stored at -20°C.

Preparation of metabolite fractions.—Blood samples were allowed to melt and were immediately extracted with chloroform (3 × 10 ml) in a separatory funnel. The samples were washed from the separatory funnel with methanol and were immediately evaporated to dryness with a vacuum pump; the residues in 10 ml of methanol were treated with 5 ml of ethereal diazomethane (prepared from Diazald, Aldrich Chemical Co, Milwaukee, WI, by the procedure supplied with the intermediate), stirred for 10 minutes at room temperature, and evaporated to dryness at water aspirator pressure. The chloroform extracts were dried over sodium sulfate and were filtered, the sodium sulfate was washed with chloroform and was filtered, and the combined filtrate and wash from each sample was evaporated at water aspirator pressure. Chloroform and methanol extract residues were stored at -20°C awaiting thin-layer chromatography.

Thin-layer chromatography.—Analysis of chloroform and methanol extracts was performed in quadruplicate on Analtech (Newark, DE) precoated silica-gel G plates (250 μm thickness) in acetone:chloroform (3:1, vol/vol) for chloroform extracts and in chloroform:methanol (9:1, vol/vol) for methanol extracts. Plates were activated by heating for 1 hour at 100°C and storing in a dessicated cabinet. Methanol extracts were analyzed on plates additionally heated 15 minutes at 140°C just prior to analysis; plates were allowed to cool to room temperature before sample application. Isolation of metabolites and radioactivity determination were accomplished as previously described (14) for each metabolite in quadruplicate for each extract. The extraction procedure has previously been shown to remove approximately 95% of the radioactivity present in either blood (6) or plasma (14).

Pharmacokinetic analysis.—The trapezoidal rule was used to measure the areas under the CPA- and metabolite-blood-decay-curves. Areas were determined by assuming that efficacious levels of CPA and its alkylating metabolites had totally cleared from mouse blood 60 minutes after treatment and from the patient's blood 24 hours after treatment (3).

RESULTS AND DISCUSSION

In the mouse, blood levels of parent drug, the nonpolar cytotoxic metabolite 4-hydroxy-CPA, the polar cytotoxic metabolite PM, the other nonpolar alkylating metabolites (4-keto-CPA, *N*-dechloroethyl-CPA, and alcophosphamide), and the other major polar alkylating metabolite carboxyphosphamide are shown in table 1. Contrary to the suggestion of Juma et al (1), no qualitative differences in the metabolite spectrum were observed between the two routes of treatment. Relatively low levels of metabolites were found 5 minutes after an iv dose, with maximum levels for both routes being attained 15-30 minutes after treatment. The 5-minute sample was collected and analyzed to determine whether high levels of metabolites would be produced in such a short time after treatment. These results are similar to those observed previously after ip administration of CPA (14,15).

Comparative levels of the proximate cytotoxic species (4-hydroxy-CPA) and the ultimate cytotoxic species (PM) are of major interest. Figure 2 shows the blood decay curve for 4-hydroxy-CPA, the levels of which are somewhat higher after iv treatment. A similar pattern was observed for PM, as shown in figure 3. The relative in vitro cytotoxicity of these two important circulating metabolites has been found to be similar against some cell lines (6) but not against some others (5,7), where 4-hydroxy-CPA was found to be more cytotoxic.

Comparative AUC data for parent drug and alkylating metabolites are shown in table 2. The overall oral/iv AUC ratio (excluding CPA) is 0.40, clearly indicating higher levels of alkylating metabolites after iv administration. Even excluding carboxyphosphamide, the oral/iv ratio is 0.60. Consequently, these results in mice contrast with results obtained by Juma et al (1) in patients. Furthermore, the data do not establish by either route a clear dominance in AUC values of either of the proposed, activated, blood transport forms of CPA, ie, 4-hydroxy-CPA or PM. The data are consistent, how-

TABLE 1.—Comparative blood levels of CPA and its alkylating metabolites in DBA/2 mice after iv vs oral administration (200 mg/kg)

	Blood levels (μg/ml ± SD) and collection times (mins)											
	5		15		30		45		60		90	
Metabolite	Iv	Oral	Iv	Oral	Iv	Oral	Iv	Oral	Iv	Oral	Iv	Oral
CPA	10.21 ± 1.51	—	11.34 ± 1.82	1.03 ± 0.21	5.41 ± 0.81	0.92 ± 0.11	1.04 ± 0.22	—	0.53 ± 0.10	0.42 ± 0.09	—	0.10 ± 0.02
4-Hydroxy-CPA	0.30 ± 0.05	—	0.31 ± 0.07	0.11 ± 0.04	0.54 ± 0.06	0.31 ± 0.05	0.32 ± 0.04	—	0.10 ± 0.03	0.10 ± 0.02	—	0.11 ± 0.01
4-Keto-CPA	0.81 ± 0.12	—	4.22 ± 0.92	0.74 ± 0.10	2.53 ± 0.33	0.71 ± 0.08	2.74 ± 0.44	—	0.82 ± 0.06	0.53 ± 0.11	—	0.23 ± 0.04
N-Dechloroethyl-CPA	0.23 ± 0.03	—	0.54 ± 0.15	0.13 ± 0.03	0.30 ± 0.07	0.33 ± 0.04	0.40 ± 0.05	—	0.11 ± 0.00	0.12 ± 0.04	—	ND* —
Alcophosphamide	0.11 ± 0.01	—	0.30 ± 0.06	0.10 ± 0.00	0.21 ± 0.05	0.40 ± 0.11	0.21 ± 0.06	—	0.11 ± 0.04	0.10 ± 0.03	—	0.12 ± 0.05
Carboxyphosphamide	0.30 ± 0.08	—	6.64 ± 0.98	0.22 ± 0.03	4.64 ± 0.79	0.41 ± 0.07	0.40 ± 0.79	—	0.50 ± 0.16	0.41 ± 0.06	—	0.11 ± 0.03
PM	0.12 ± 0.04	—	0.51 ± 0.09	0.11 ± 0.02	0.40 ± 0.04	0.30 ± 0.03	0.20 ± 0.03	—	0.04 ± 0.01	0.11 ± 0.03	—	0.03 ± 0.01

*ND = not detected with NBP.

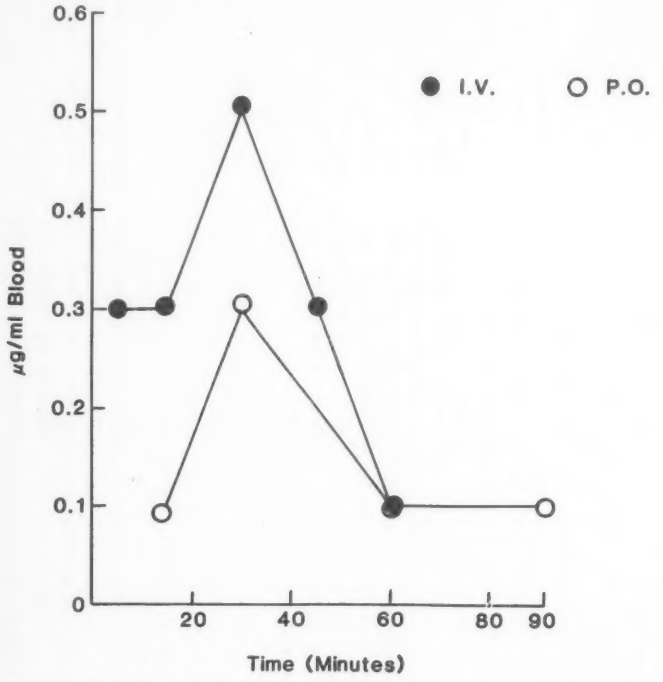

FIGURE 2.—Blood levels of 4-hydroxy-CPA in mice after iv vs oral (P.O.) administration (200 mg/kg).

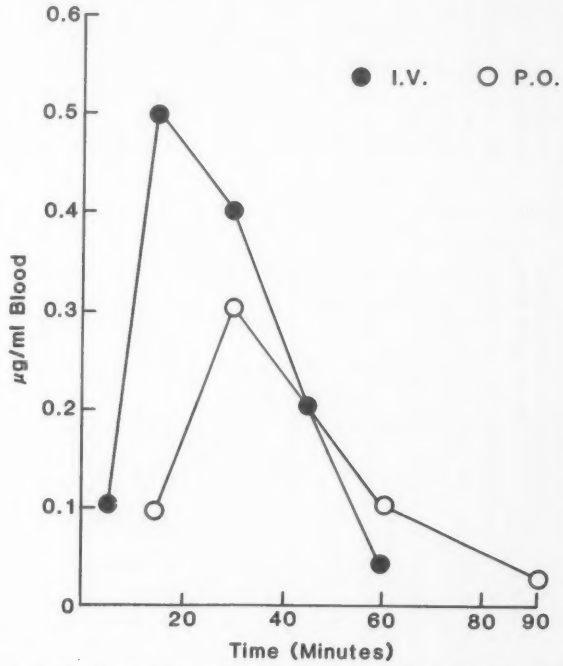

FIGURE 3.—Blood levels of PM in mice after iv vs oral (P.O.) administration (200 mg/kg).

TABLE 2.—AUC data (μg·min/ml) for CPA and its alkylating metabolites in DBA/2 mice after iv vs oral administration (200 mg/kg)

Metabolite	Iv	Oral	Oral/iv
CPA	322	55	0.17
4-Hydroxy-CPA	24	18	0.75
4-Keto-CPA	187	97	0.52
N-Dechloroethyl-CPA	25	11	0.44
Alcophosphamide	17	22	1.30
Carboxyphosphamide	247	48	0.19
PM	18	13	0.72
Totals (excluding CPA)	518	209	0.40

ever, with somewhat increased levels of cytotoxic factor(s) (oral/iv ratio = 0.75 for 4-hydroxy-CPA and 0.72 for PM) in a mouse spleen colony assay, in which the surviving fraction of colony-forming units per femur was 0.098 after oral treatment versus 0.035 after iv treatment at a dose of 200 mg/kg (16).

Studies in a single patient were conducted to determine the suitability of the methods and to obtain preliminary data on comparative blood levels of 4-hydroxy-CPA and PM after oral or iv treatment with CPA. These data (table 3) do not indicate the differences in oral/iv AUC ratios observed in the mouse study, but suggest essentially equivalent ratios for total alkylating metabolites and for both 4-hydroxy-CPA and PM. Despite widespread clinical use of CPA, very limited data exist on relative AUCs for 4-hydroxy-CPA and PM in the same patient. Jardine et al (3) and Juma et al (1) determined PM levels in patients after iv or oral treatment or both. Wagner et al (17) determined 4-hydroxy-CPA in patients after iv treatment in one study and 4-hydroxy-CPA and a fraction containing PM in patients after iv treatment in another study (4). Jardine et al (3) observed high AUC values for PM after iv treatment with 60–75 mg/kg of CPA, whereas Wagner et al (17) observed relatively low AUC values for 4-hydroxy-CPA after an iv dose of 20 mg/kg. Our data are more in agreement with the AUC values reported by Wagner et al (17) and resulted from an essentially identical dose. Our investigation in one patient is the only study of simultaneous determination of 4-hydroxy-CPA and PM alone in the same patient after either iv or oral drug treatment. We plan to study additional patients to establish statistically significant AUC data for these two important metabolites of CPA.

The difference in AUC values between mice and humans, as observed in our studies, is not surprising in view of the appreciable species differences reported for CPA metabolism. These differences are believed to reflect primarily variation in metabolism by hepatic microsomes (18). The reduced bioavailability of CPA and its metabolites in mice after oral administration may result from increased fecal excretion; this may explain the large difference in oral/iv ratio for total alkylating metabolites in mice (0.40), but not in man, where the ratio is 0.87.

TABLE 3.—AUC data (μg·hr/ml) for CPA and its major metabolites in man after iv vs oral administration (1 g)

Metabolite	Iv	Oral	Oral/iv
CPA	21.9	19.5	0.89
4-Hydroxy-CPA	1.2	1.0	0.83
4-Keto-CPA	1.3	1.3	1.00
Carboxyphosphamide	12.0	10.5	0.88
PM	1.2	0.9	0.75
Totals (excluding CPA)	15.7	13.7	0.87

REFERENCES

1. JUMA FD, ROGERS HJ, and TROUNCE JR. Pharmacokinetics of cyclophosphamide and alkylating activity in man after intravenous and oral administration. Br J Clin Pharmacol 8:209–217, 1979.
2. COLVIN M, PADGETT CA, and FENSELAU C. A biologically active metabolite of cyclophosphamide. Cancer Res 33:915–918, 1973.
3. JARDINE I, FENSELAU C, APPLER M, ET AL. Quantitation by gas chromatography-chemical ionization mass spectrometry of cyclophosphamide, phosphoramide mustard, and nornitrogen mustard in the plasma and urine of patients receiving cyclophosphamide therapy. Cancer Res 38:408–415, 1978.
4. WAGNER T, HEYDRICH D, VOELCKER G, ET AL. Uber blutspiegel und urinausscheidung von aktiviertem cyclophosphamid und seinen deaktivierungsprodukten beim menschen. J Cancer Res Clin Oncol 96:79–92, 1980.
5. DOMEYER BE, and SLADEK NE. Kinetics of cyclophosphamide biotransformation in vivo. Cancer Res 40:174–180, 1980.
6. STRUCK RF, KIRK MC, WITT MH, ET AL. Isolation and mass spectral identification of blood metabolites of cyclophosphamide: evi-

dence for phosphoramide mustard as the biologically active metabolite. Biomed Mass Spectrom 2:46-52, 1975.
7. BROCK N, and HOHORST H-J. The problem of specificity and selectivity of alkylating cytostatics: studies on *N*-2-chloroethylamide-oxazaphosphorines. Z Krebsforsch 88:185-215, 1977.
8. COX PJ, PHILLIPS BJ, and THOMAS P. The enzymatic basis of the selective action of cyclophosphamide. Cancer Res 35:3755-3761, 1975.
9. FENSELAU C, KAN M-NN, RAO SS, ET AL. Identification of aldophosphamide as a metabolite of cyclophosphamide in vitro and in vivo in humans. Cancer Res 37:3738-3741, 1977.
10. FRIEDMAN OM, WODINSKY I, and MYLES A. Cyclophosphamide (NSC-26271)-related phosphoramide mustards—recent advances and historical perspective. Cancer Treat Rep 60:337-346, 1976.
11. FENSELAU C, LEHMAN JP, MYLES A, ET AL. Iminocyclophosphamide as a chemically reactive metabolite of cyclophosphamide. Drug Metab Dispos 10:636-640, 1982.
12. STRUCK RF, KIRK MC, MELLETT LB, ET AL. Urinary metabolites of the antitumor agent cyclophosphamide. Mol Pharmacol 7:519-529, 1971.
13. BAKKE JE, FEIL VJ, FJELSTUL CE, ET AL. Metabolism of cyclophosphamide by sheep. J Agric Food Chem 20:384-388, 1972.
14. ALBERTS DS, PENG YM, CHEN HS, ET AL. Effect of phenobarbital on plasma levels of cyclophosphamide and its metabolites in the mouse. Br J Cancer 38:316-322, 1978.
15. HIPKENS JH, STRUCK RF, and GURTOO HL. Role of aldehyde dehydrogenase in the metabolism-dependent biological activity of cyclophosphamide. Cancer Res 41:3571-3583, 1981.
16. STRUCK RF, EL DAREER SM, ALBERTS DS, ET AL. Comparative pharmacokinetics of cyclophosphamide and its alkylating metabolites in mouse blood after intravenous or oral administration. Proc Am Assoc Cancer Res and ASCO 22:210, 1981.
17. WAGNER T, HEYDRICH D, JORK T, ET AL. Comparative study on human pharmacokinetics of activated ifosfamide and cyclophosphamide by a modified fluorometric test. J Cancer Res Clin Oncol 100:95-104, 1982.
18. FRIEDMAN OM, MYLES A, and COLVIN M. Cyclophosphamide and related phosphoramide mustards, current status and future prospects. *In* Advances in Cancer Chemotherapy (Rosowsky A, ed). New York, Marcel Dekker, 1979.

Cisplatin, Doxorubicin, Cyclophosphamide, Lomustine, and Vincristine (PACCO) in the Treatment of Non-Small Cell Bronchogenic Carcinoma[1]

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A total of 43 patients with non-small cell carcinoma from a small preliminary trial and a larger in-house study were evaluated after treatment with cisplatin (50 mg/m²), doxorubicin (50 mg/m²), cyclophosphamide (300 mg/m²), and vincristine (1.4 mg/m²), all given iv on Day 1, and lomustine (50 mg/m²) given orally on Day 1. The response rate in the larger trial was 9%, with a 95% confidence interval of 2%–24%. For the combined group of all patients, median survival was 200 days, with a 95% confidence interval of 115–229 days. Hematologic toxicity and nausea and vomiting were moderate to severe and there were two treatment-related deaths. This combination drug regimen appears to have no advantages over other, less toxic regimens in the treatment of non-small cell bronchogenic carcinoma. [Cancer Treat Rep 68:771–773, 1984]

Combination chemotherapy for non-small cell carcinoma of the lung has shown limited effectiveness, with typical response rates of 22%–48% for various combination drug regimens (1–3). A recent report of responses in 23 of 35 patients (65.7%) with non-small cell lung cancer using cisplatin, doxorubicin, cyclophosphamide, lomustine, and vincristine (PACCO) (4) prompted our investigation of this regimen. This paper reports our experience with PACCO.

METHODS AND MATERIALS

Following the standard design for phase II trials (5), a preliminary trial of PACCO was undertaken starting in September 1978. Because of the favorable responses noted in this small trial, an in-house study (CO 7951) was begun. By March 1981, a total of 46 eligible patients with histologically documented non-small cell lung carcinoma incurable by surgery or radiation therapy had been treated. Patients were required to be $\leq$ 70 years of age, have measurable or evaluable disease, and have an Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2. Creatinine clearance had to be $\geq$ 50 ml/minute and there could be no history of heart disease. Previous chemotherapy or radiation therapy did not render a patient ineligible, provided there had been documented disease progression since treatment.

The PACCO regimen consisted of 50 mg/m² of cisplatin, 50 mg/m² of doxorubicin, 300 mg/m² of cyclophosphamide, and 1.4 mg/m² (not to exceed 2.0 mg) of vincristine, all given iv on Day 1, and 50 mg/m² of lomustine given orally on Day 1. Treatment was repeated in 4-week cycles. Cisplatin was given with hydration and mannitol and furosemide-induced diuresis similar to the regimen of Vogel et al (6). Appropriate dosage modifications were made for changes in renal or hepatic function or decreased wbc and platelet counts.

Standard ECOG criteria for solid tumor response were used to evaluate all patients. Measurement parameters had to be present for at least two measurement periods that were separated by at least 4 weeks to be considered a response.

Confidence intervals for median survival times are based on the method of Brookmeyer and Crowley (7). Exact distributions are used to construct confidence intervals and tests for response rates; in tests, complete response (CR) is scored as 2, partial response (PR) as 1, and all others as 0.

RESULTS

A total of 52 patients were entered in the study. Six patients were not eligible: two did not have lung tumors, one had insufficient information to indicate eligibility,

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one had no disease outside a previous radiation field, one was given low doses of medication, and one was too old. Three additional patients were not evaluable. One patient refused treatments prior to being in the study long enough for evaluability, one had inadequate data, and one was lost to follow-up. Thus, there were 43 patients eligible and evaluable in the study. There were 24 patients with squamous cell carcinoma, nine with large cell carcinoma, and ten with adenocarcinoma. Ten, 24, and nine patients had a performance status of 0, 1, and 2, respectively. Twenty-seven patients had received no previous treatment, 12 had had previous radiation therapy, one had had previous chemotherapy, and three had had previous irradiation and chemotherapy.

Response is shown in table 1. The patients in the small preliminary trial and in the larger in-house study did appear to differ with respect to response rates (preliminary trial, 33%; follow-up trial, 9%; one-sided P value = 0.054). These differences may be due to chance, to regression towards the mean (an unavoidable difficulty with the standard design), or to other causes. It would seem prudent to us to assume that the response rate from the larger in-house protocol (9% with 95% confidence interval of 2%-24%) is closest to the true response rate.

Toxicity

Hematologic toxicity was moderate, with two patients having wbc count nadirs < 1000/mm^3 and three having platelet count nadirs < 25,000/mm^3. Nausea and vomiting was often severe, with eight patients having intractable vomiting despite use of routine antiemetics. There were two deaths thought to be treatment-related. Both deaths occurred approximately 2 weeks after the first treatment when chest x-ray revealed infiltrates and patients had wbc counts of 1500 and 1300/mm^3, respectively. The second patient also had severe anemia, thrombocytopenia, and cardiac arrhythmias. Autopsies were not done on either patient.

Survival and Disease Progression

The median survival and progression times are shown in table 1. Of the six responders, four were alive when last seen at 562, 525, 524, and 480 days. One responder died at 543 days and another at 200 days. Thus, responders generally lived longer than the median survival time for the entire group.

DISCUSSION

In the largest of our two groups, the response rate to PACCO was 9% (95% confidence interval, 2%-24%), far below that reported by Takita et al (4). This figure is also less than that reported for the most active combinations in non-small cell lung cancer. The overall median survival of the patients treated with PACCO, 200 days (95% confidence interval; 115-229 days), was similar to overall survival figures reported for other lung cancer regimens. In the study of PACCO by Takita et al (4), the overall median survival was estimated to be 47.5 weeks or 332.5 days; again, we were unable to duplicate these results. Although in the study by Takita et al there were 19 of 35 patients with disease limited to the hemithorax compared to five of 39 with "only lung primary" as their dominant disease in our group, this alone cannot explain the discrepancy in response or survival, since even their patients with disseminated disease showed ten of 16 (62.5%) patients responding and an estimated survival of 29.5 weeks or 206.5 days, about the median survival of our total group of patients.

Because of the small number of patients in our study, results obtained by analyzing various subgroups would appear to be tenuous. The fact that all six responding patients survived longer than the overall median survival may be due to selection of a more favorable group of patients because of known or unknown factors (8). It should be noted that there does seem to be a degree of activity for this combination, since one CR was achieved. This pa-

TABLE 1.—Response to PACCO*

Response	No. of patients responding (%)		
	Preliminary trial	Follow-up trial†	Both trials‡
CR	1 (11)	0 (0)	1 (2)
PR	2 (22)	3 (9)	5 (12)
No change	1 (11)	13 (38)	14 (33)
Disease progression	5 (56)	16 (47)	21 (49)
Drug death	0 (0)	2 (6)	2 (5)
Totals	9	34	43

*For both trials combined, median survival time was 200 days (range, 115-229) and median time to disease progression was 63 days (range, 38-109).

†95% confidence interval for the response rate (CR + PR) is 2%-24%.

‡95% confidence intervals for the response rates were not done, because the 1-tailed P value for comparing response rates of each trial is 0.05. Results cannot be safely pooled.

tient eventually relapsed intracranially and died from his tumor.

In summary, it would seem that the PACCO regimen has minimal effect in non-small cell bronchogenic carcinoma. Response rates appear to be no better than those with other combination drug regimens and, with two treatment-related deaths, toxicity was greater. We suggest that new and different drug regimens hold more promise for successful treatment of patients with non-small cell bronchogenic carcinoma.

REFERENCES

1. BITRAN JD, DESSER RK, DEMEESTER TR, ET AL. Cyclophosphamide, adriamycin, methotrexate, and procarbazine (CAMP)—effective four-drug combination chemotherapy for metastatic non-oat cell bronchogenic carcinoma. Cancer Treat Rep 60:1225–1230, 1976.
2. EAGAN RT, INGLE JN, FRYTAK S, ET AL. Platinum-based polychemotherapy versus dianhydrogalactitol in advanced non-small cell lung cancer. Cancer Treat Rep 61:1339–1345, 1977.
3. CREECH RH, MEHTA CR, COHEN M, ET AL. Results of a phase II protocol for evaluation of new chemotherapeutic regimens in patients with inoperable non-small cell lung carcinoma (EST-2575, generation I). Cancer Treat Rep 65:431–438, 1981.
4. TAKITA H, MARABELLA PC, EDGERTON F, ET AL. *cis*-Dichlorodiammineplatinum(II), adriamycin, cyclophosphamide, CCNU, and vincristine in non-small cell lung carcinoma: a preliminary report. Cancer Treat Rep 63:29–33, 1979.
5. GEHAN EA. The determination of the number of patients required in a preliminary and a follow-up trial of a new chemotherapeutic agent. J Chronic Dis 13:346–353, 1961.
6. VOGEL SE, ZARAVINOS T, and KAPLAN BH. Toxicity of *cis*-diamminedichloroplatinum II given in a two-hour outpatient regimen of diuresis and hydration. Cancer 45:11–15, 1980.
7. BROOKMEYER R, and CROWLEY J. A confidence interval for the median survival time. Biometrics 38:29–43, 1982.
8. AISNER J, and HANSEN HH. Commentary: Current status of chemotherapy for non-small cell lung cancer. Cancer Treat Rep 65:979–986, 1981.

Oral Etoposide in Gestational Trophoblastic Disease[1]

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Ten patients with persistent or metastatic trophoblastic disease following molar pregnancy were treated with etoposide orally, administered on an outpatient basis. All patients achieved sustained biochemical remission. The mean duration of treatment was 8 weeks. The side effects were mild. The use of etoposide in patients resistant to conventional chemotherapy is discussed. [Cancer Treat Rep 68:775–777, 1984]

Etoposide is a semisynthetic derivative of podophyllotoxin and has been shown to have antitumor activity against gestational choriocarcinoma (1). Newlands and Bagshawe (1) reported a combined response and improvement rate of 64% in patients resistant to the multiple-agent regimen CHAMOMA (hydroxyurea, methotrexate, cyclophosphamide, dactinomycin, vincristine, and doxorubicin).

MATERIALS AND METHODS

The ten patients entered in this study had either persistent or metastatic gestational trophoblastic disease. They were categorized into low-, medium-, and high-risk groups using a scoring system taking into consideration various prognostic factors: type of antecedent pregnancy; interval between antecedent pregnancy and start of chemotherapy; pretreatment human chorionic gonadotropin (hCG) level; history of previous chemotherapy; and number, size, and sites of metastases. One patient had had total hysterectomy for massive hemorrhage; the histological diagnosis was choriocarcinoma. Nine patients belonged to the low- or medium-risk group. The trophoblastic disease was confined to the uterus or had metastasized to the lungs or pelvis. One patient had metastases to the lungs, vagina, and liver; she belonged to the high-risk group. None of the patients had had prior chemotherapy. The age range of the patients was from 16 to 52 years. The duration of their disease from the start of treatment was < 4 months. Their initial urinary hCG level was < 100,000 IU/24 hours, and their serum β-hCG level was < 10^4 mIU/ml. All of the patients developed the disease following molar pregnancy, one of whom had a partial mole. All of the histological slides from the uterine curettage were reviewed by the same pathologist, and the histological diagnoses were confirmed.

Pretreatment Evaluation

The pretreatment evaluation of the patients included complete physical examination, chest x-ray, pelvic and hepatic arteriograms, liver and uterus sonograms, cbc, and liver and renal function tests. Serial serum hCG and 24-hour urinary hCG were estimated. Lumbar puncture was performed and cerebrospinal fluid examined for chemistry, cytology, culture, and hCG titer. Repeat dilation and curettage was not routinely performed.

Treatment Protocol and Monitoring

Etoposide was administered orally at a dose of 200 mg/m^2 for 5 consecutive days. The course was repeated after 1 week, and the drug was given on an outpatient basis. The patients were seen weekly for detection of side effects and also for monitoring of response to chemotherapy.

All patients were monitored twice weekly by radioimmunoassay for total hCG and serum β-hCG. The sensitivity of the β-hCG assay was 5 mIU/ml. During the course of treatment, hemoglobin, leukocyte, and platelet counts were done twice weekly to detect any marrow depression. Liver and renal function tests and chest x-ray were done every 2 weeks. The patients were examined for dermatological, gastrointestinal, and neurological symptoms.

The trial would be stopped if the patient showed any untoward reaction, eg, anaphylaxis, or if there was no evidence of response to chemotherapy after 4 weeks of treatment. Otherwise, oral etoposide was given until the

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patient achieved complete biochemical remission. Complete biochemical remission. Complete biochemical remission is defined as the achievement of a β-hCG level of < 5 mIU/ml for 3 consecutive weeks. Duration of treatment after normalization of hCG titer was determined by extrapolating the hCG regression pattern to 10^{-4} log.

RESULTS

Characteristics of the ten patients are summarized in table 1. All patients responded well to oral etoposide and achieved sustained biochemical remission. The disappearance of lung metastases coincided with the induction of biochemical remission. The mean number of courses needed to induce biochemical remission was 2.7. The time interval between start of chemotherapy and induction of biochemical remission was between 2 and 8 weeks (mean, 4.2). The duration of chemotherapy varied from 4 to 12 weeks (mean, 8 [2.36 SD]).

The most prominent nonhemotological toxic effect was temporary alopecia. Seven patients developed total alopecia after the second or third course of chemotherapy and three developed partial alopecia during treatment. Eight patients experienced mild nausea and anorexia. Only two patients had vomiting and required antiemetic therapy. Two patients developed mild stomatitis. One patient developed pigmentation of the nail beds and fingers; the pigmentation disappeared 2 months after chemotherapy. No hypotension, peripheral neuropathy, nephrotoxicity, or hepatotoxicity was noted.

Hematological toxic effects were mild and reversible. Four patients developed leukopenia. In two of these patients the leukocyte count nadirs were 2500 and 2700/mm^3, respectively. The other two patients had very mild leukopenia; the leukocyte count never decreased to < 3000/mm^3. None of these patients developed any complications from leukopenia. The duration of leukopenia was transient, lasting for only 3–4 days. Chemotherapy was not delayed in any of these patients, and there was no evidence of cumulative myelosuppression. Thrombocytopenia (platelet count < 100 × 10^9/L) was absent (table 2).

Three patients experienced amenorrhea during chemotherapy. Two patients recovered and had spontaneous menstrual cycles 6 months after stopping the chemotherapy. One patient had persistent amenorrhea. Repeated hormonal profile studies showed persistently raised serum follicle stimulating hormone and luteinizing hormone and low serum E_2 levels. Ovarian failure was diagnosed. All three patients belonged to the older age group. The patient who developed ovarian failure after chemotherapy was 52 years old. The other two patients were 40 and 41 years old, respectively. There was no evidence of ovarian suppression in the young patients.

The follow-up period in these patients was 12–21 months. No evidence of disease was detected. One patient became pregnant 1 year after complete biochemical remission.

DISCUSSION

The effectiveness of oral administration of etoposide as a primary chemotherapeutic agent in gestational trophoblastic disease has been demonstrated in this study. All of the patients achieved sustained biochemical remission. The duration of remission ranged from 12 to 21 months (median, 13.5) (table 1). The mean number of courses required for induction of biochemical remission was similar to that of the low-risk group treated with a methotrexate-leucovorin rescue regimen. The duration of chemotherapy was also comparable. Etoposide is more expensive than the methotrexate-leucovorin regimen. This is compensated by a shortened hospital stay. Most of these patients preferred etoposide given on an outpatient basis to methotrexate which was usually administered in the hospital. The disruption of family life was much less in

TABLE 1.—Status of patients before therapy

Patient No.	Age (yrs)	Parity	Duration of disease (mos)	Initial β-hCG (mIU/ml)	Uterine lesion*	Metastasis	Total No. of courses	Length of remission (mos)	Disease status†
1	24	1	2	1800	+	None	3	12	NED
2	47	3	1	300	Chorio-carcinoma	None	4	13	NED
3	27	1	1½	5000	+	Lungs, vagina, liver	7	21	NED
4	49	6	3	600	+	-	5	17	NED
5	24	1	1½	400	+	-	3	21	NED
6	26	3	2	4500	+	-	5	14	NED
7	41	4	1½	20,000	+	-	8	13	NED
8	52	8	4	200	+	-	3	13	NED
9	40	2	4	200	+	-	4	12	NED
10	16	1	2	2000	+	-	4	20	NED

* + = vascular uterine tumor detected by pelvic angiography. Choriocarcinoma proven by histology; hysterectomy was performed.
†NED = no evidence of disease.

TABLE 2.—Toxic effects of oral etoposide

Toxic effect	No. of patients
Nausea	
Mild	8
Moderate	2
Vomiting	2
Mild stomatitis	2
Alopecia	10
Leukopenia	
Mild*	2
Moderate†	2
Amenorrhea	3
Pigmentation of nail bed	1

*< 3000 cells/mm^3.
†Nadir, 2500 cells/mm^3.

the group receiving oral etoposide. Patient compliance was good; none of the patients defaulted during treatment.

Etoposide may be an alternative to the methotrexate-leucovorin regimen as a first-line drug, especially in patients with impaired liver function. This may be a better alternative than dactinomycin. Most of our patients dislike the administration of dactinomycin because of its severe gastrointestinal toxicity.

Since etoposide has proven effective in gestational trophoblastic disease, it may be a useful drug for patients who are resistant to conventional drugs. It can also be incorporated into combination-chemotherapy regimens for the high-risk patients. Newlands first reported its use in patients resistant to combination therapy (Bagshawe's regimen) in 1980. We had used etoposide in three patients who were resistant to Bagshawe's regimen. All patients achieved sustained biochemical remission; the duration of biochemical remission was 13, 25, and 39 months, respectively. Etoposide should be included in the combination-therapy regimen, especially for patients who fail to respond to the conventional therapy.

REFERENCE

1. NEWLANDS ES, and BAGSHAWE KD. Anti-tumour activity of the epipodophyllin derivation VP16-213 (etoposide NSC 141540) in gestational choriocarcinoma. Int J Cancer 16:401-405, 1980.

Treatment of Malignant Germ Cell Tumors of the Ovary With Cisplatin, Vinblastine, and Bleomycin (PVB)[1]

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Seven patients with germ cell tumors of the ovary were treated with four cycles of cisplatin, vinblastine, and bleomycin. Six patients were without evidence of disease 17+ to 37+ months after completion of the remission-induction chemotherapy, and one patient relapsed after 9 months. Substantial toxicity of the cisplatin, vinblastine, and bleomycin regimen was noted. Despite the high response rate suggested in this small series, the optimal chemotherapeutic treatment for this disease has yet to be determined. [Cancer Treat Rep 68:779–781, 1984]

Germ cell tumors comprise 5% of all malignant ovarian tumors. Surgery with or without radiotherapy has limited value, with the exception of that for pure dysgerminomas, but chemotherapy following surgery leads to an increase in disease-free survival (1). Since the introduction of cisplatin in the treatment of patients with germ cell tumors of the testis, a 70%–80% cure rate has been achieved (2). We present the clinical outcome of seven patients with malignant germ cell tumors of the ovary treated with cisplatin, vinblastine, and bleomycin (PVB) according to Einhorn and Donohue (2).

PATIENTS AND METHODS

From 1979 to 1982, seven consecutive patients with disseminated germ cell tumors of the ovary were admitted to the University Hospital of Groningen, The Netherlands. Stage was defined according to the International Federation of Gynecology and Obstetrics (FIGO) classification. Alpha-fetoprotein in serum was assayed by means of a radioimmunoassay using a commercial radioimmunoassay kit (Behring Werke AG, Marburg, W Germany).

Patient characteristics are summarized in table 1. No patients had received radiotherapy, but one patient (No. 5) had been treated previously with 5-FU, dactinomycin, and cyclophosphamide for 2 years. Two patients (Nos. 1 and 4) were treated because of relapse 5 and 19 months, respectively, after complete resection of a FIGO stage I tumor. The other patients started PVB treatment within 1 month after diagnosis. All patients were treated with a combination of cisplatin (20 mg/m^2 infused over 4 hours for 5 consecutive days with pre- and post-hydration), vinblastine (0.2 mg/kg by iv push on Days 1 and 2 of each cycle), and bleomycin (30 mg iv on Days 2, 9, and 16). Four cycles were administered. Dose modifications were not required for myelotoxicity, but bleomycin was withheld from two patients because of pulmonary toxicity. All patients were clinically re-evaluated after completion of the remission-induction treatment. Second-look laparotomy was part of the standard restaging procedure and included resection of all visible tumor residue and/or biopsy of all previously tumor-bearing areas. Second-look laparotomy was refused by Patients 3 and 5.

After restaging, three patients were maintained on treatment with vinblastine alternating with vinblastine plus cisplatin at 3-week intervals for 1 year.

RESULTS

Five patients (71%) achieved a CR. In the two patients (29%) who achieved PR, surgery revealed benign differentiation of the tumor. In one patient (No. 7) the disease recurred 9 months after a surgically confirmed CR. The other patients have been followed for 17+ to 37+ months after completion of induction chemotherapy and have remained disease-free. Toxicity from the PVB treatment is substantial (2). All patients experienced nausea, vomiting, weight loss, alopecia, skin pigmentation, myalgia, paresthesia, and subileus. Hematological toxicity is

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TABLE 1.—Patient characteristics at the start of PVB chemotherapy and results of remission induction

Patient No.	Age (yrs)	Diagnosis*	Stage (FIGO)	α-fetoprotein (μg/L)	Remission†	Surgical re-evaluation‡	Maintenance therapy	Disease-free survival (mos)§
1	22	IT, grade 3	IV	615	PR	MT	Yes	30+
2	17	IT + E + EDS	II	1200	PR	MT	Yes	29+
3	34	D + E + IT, grade 2	III	10,000	CR	Not done	Yes	24+
4	23	D	IV	5	CR	Necrosis	No	17+
5	22	EDS	II	2050	CR	Not done	No	37+
6	22	EDS	I	6400	CR	Necrosis	No	21+
7	23	EDS	IV	26,000	CR	Necrosis	No	9+

*IT = immature teratoma; E = embryonal carcinoma; EDS = endodermal sinus tumor; and D = dysgerminoma.
†PR = partial remission; CR = complete remission.
‡MT = mature teratoma.
§From completion of the remission-induction chemotherapy.

described in table 2. Septicemia was documented in only one patient (No. 5) with a wbc count of 0.4 × 10^9/L. In two patients (Nos. 1 and 5) signs and symptoms of bleomycin-induced interstitial pneumonitis were found after 330 and 240 mg of bleomycin, respectively. Patient 7 experienced severe hyponatremia (119 mmols/L of sodium) during the first and third cycles, accompanied by hypertension and seizures. Neither elevations of serum creatinine nor allergic reactions to the anticancer drugs used were recorded for these patients.

DISCUSSION

The optimal approach to treatment of ovarian germ cell tumors, with the exception of dysgerminoma, has not been completely elucidated. Initially, localized germ cell tumors of the ovary usually recur within 1 year after surgery with or without radiotherapy or single-agent chemotherapy (1,3). Combination chemotherapy increases disease-free survival and probably cures a significant number of patients (1). The cure rate of the older combinations including vincristine, dactinomycin, cyclophosphamide, and/or antimetabolites can be estimated to be approximately 50% (1,3-8). When used as adjuvant to complete tumor resection, these regimens achieve even better results (1,8).

Based on the considerable success rate of the Einhorn regimen in testicular germ cell tumors, a similar combination has been applied in our patients, leading to long-term remissions in six of seven patients despite advanced disease. Others have also used this combination chemotherapy. Of 12 nonpretreated patients (9-12), ten completely responded to PVB and survived for 2+ to 19+ months. In a preliminary report by Williams et al (13), results from the largest series of patients yet studied suggested a high, surgically proven CR rate (ten of 13 patients), but no definite conclusions can be drawn from that study because of short follow-up and the abstract form of the report.

TABLE 2.—Hematological toxicity during remission-induction chemotherapy (28 cycles)

Hematological toxicity	No. of cycles
Wbc count nadir*	
≥ 4.0	0
3.0-3.9	2
2.0-2.9	1
1.0-1.9	10
< 1.0	15
Platelet count nadir*	
≥ 100	6
75-79	6
50-74	10
25-49	6
< 25	0

* × 10^9 cells/L.

The PVB remission-induction regimen has an established role in the treatment of testicular germ cell tumors, and maintenance therapy has been shown to have no value (14). Despite the good results initially obtained in patients with ovarian germ cell tumors, it seems too early to accept PVB or other platinum-containing regimens as superior to other forms of combination chemotherapy. Although the cumulative result of the platinum-containing regimens in non-pretreated patients is impressive, only a prospective study comparing the toxicity and the efficacy of the various regimens can define the value of each in this disease.

REFERENCES

1. CURRY SL, SMITH JP, and GALLAGHER HS. Malignant teratoma of the ovary: prognostic factors and treatment. Am J Obstet Gynecol 131:845-850, 1978.
2. EINHORN LH, and DONOHUE JP. Combination of chemotherapy in disseminated testicular cancer: the Indiana University experience. Semin Oncol 6:87-93, 1979.
3. KURMAN RJ, and NORRIS HJ. Endodermal sinus tumor of the ovary: a clinical and pathological analysis of 71 cases. Cancer 38:2404-2419, 1976.
4. CANGIR A, SMITH JP, and VAN EYS J. Improved prognosis in children with ovarian cancers following modified VAC (vincristine sulfate, dactinomycin and cyclophosphamide) chemotherapy. Cancer 42:1234-1238, 1978.
5. SLAYTON RE, HRESHCHYSHYN MM, SILVERBERG SG, ET AL. Treatment of malignant ovarian germ cell tumors: response to vincristine, dactinomycin and cyclophosphamide (preliminary report). Cancer 42:390-398, 1978.
6. GALLION H, VAN NAGELL JR, POWELL DF, ET AL. Therapy of endodermal sinus tumor of the ovary. Am J Obstet Gynecol 135:447-452, 1979.
7. KARLEN JR, and KASTELIC JE. Endodermal sinus tumor of the ovary: an improving prognosis. Gynecol Oncol 10:206-216, 1980.
8. CREASMAN WT, FETTER BF, HAMMOND CB, ET AL. Germ cell malignancies of the ovary. Obstet Gynecol 53:226-230, 1979.
9. JULIAN CG, BARRET JM, RICHARDSON RL, ET AL. Bleomycin, vinblastine and cisplatinum in the treatment of advanced endodermal sinus tumor. Obstet Gynecol 56:396-401, 1980.
10. LOKEY JL, BAKER JJ, PRICE NA, ET AL. Cisplatin, vinblastine and bleomycin for endodermal sinus tumor of the ovary. Ann Intern Med 94:56-57, 1981.
11. JACOBS AJ, HARRIS M, DEPPE G, ET AL. Treatment of recurrent and persistent germ cell tumors with cisplatin, vinblastine and bleomycin. Obstet Gynecol 59:129-132, 1982.
12. WILTSHAW E. Germ cell tumors in females. *In* Germ Cell Tumors (Anderson CK, Jones WG, Milfod Ward A, eds). London, Taylor and Francis, 1981, pp 179-189.
13. WILLIAMS S, SLAYTON R, SILVERBERG S, ET AL. Response of malignant ovarian germ cell tumors to cisplatinum, vinblastine and bleomycin (PVB). Proc Am Assoc Cancer Res and ASCO 22:463, 1981.
14. EINHORN LH, WILLIAMS SD, TRONER T, ET AL. The role of maintenance therapy in disseminated testicular cancer. N Engl J Med 305:727-731, 1981.

Treatment of Esophageal Cancer With Vindesine: An Open Trial[1]

W. R. Bezwoda,* D. P. Derman, A. Weaving, and M. Nissenbaum[2]

Fifty-two patients with advanced esophageal cancer have been entered in an open study with vindesine. The regimen consisted of vindesine at a dose of 3 mg/m² as a continuous infusion over 48 hours followed by 3 mg/m² iv weekly for 4 weeks and then by monthly maintenance therapy using the same dose. Objective response was seen in 14 (27%) patients. Patients who responded to treatment had significant prolongation of survival. Major pretreatment prognostic factors included performance status and serum albumin concentration. It is concluded that vindesine has definite, although limited, activity against esophageal cancer. [Cancer Treat Rep 68:783–785, 1984]

Recurrent and/or inoperable esophageal cancer has an extremely poor prognosis, particularly in patients of low socioeconomic status who frequently present with severe nutritional deficit and diminished performance status together with advanced disease. Relatively few chemotherapeutic trials have been reported in this condition but previous studies include treatment schedules with bleomycin, methotrexate, cisplatin, and mitomycin (1–3). The results, however, have not been encouraging and reported response rates generally do not exceed 20%. Preliminary data from phase II trials indicated that vindesine has activity not shared by the other vinca alkaloids against a variety of cancers including esophageal carcinoma (4,5). Following a pilot study, a formal open trial of vindesine in esophageal cancer was undertaken.

The purpose of the study was to determine the response rate and duration of survival of patients with advanced esophageal cancer treated with vindesine.

PATIENTS AND METHODS

Fifty-two patients with inoperable, recurrent, and/or metastatic esophageal cancer at the Oncology Clinic of the Johannesburg Hospital were studied. The diagnosis was confirmed by histologic examination in all patients; all who were entered in the study had disease which was measurable either clinically or by radiologic methods. Patients who had received radiation therapy were excluded unless there was disease present outside previous radiation therapy fields. Patient characteristics are shown in table 1.

The therapy schedule consisted of vindesine at a dose of 3 mg/m² iv weekly × 4 weeks. The first dose of vindesine was given over 48 hours as a continuous infusion, while subsequent doses were given by iv push. This administration schedule was dictated by the need to continue treatment on an outpatient basis. Patients who received a minimum of two doses of chemotherapy are included in the analysis. Maintenance therapy was given to patients who had either disease stabilization or objective response at Week 4; therapy consisted of vindesine (3 mg/m²) given by iv push at four weekly intervals and was continued for as long as response or disease stabilization was maintained. Doses were modified according to the following hematologic values: 100% dose with wbc count > 4000/mm³ and platelet count > 100,000/mm³; 75% dose with wbc count 3000–3999/mm³ and platelet count > 75,000–90,999/mm³; and 50% dose with wbc count 2000–2999/mm³ and platelet count 50,000–74,999/mm³. With a wbc count < 2000/mm³ and a platelet count < 50,000/mm³ therapy was delayed until hematologic recovery. Response to treatment was evaluated using accepted methods of objective assessment, and toxicity was monitored clinically and by means of appropriate blood tests.

RESULTS

Fifty-one of the 52 patients entered in the study are

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Mean age in yrs: 53 ± 8	
Sex	
Male	35
Female	17
Disease sites	
Esophagus	40
Lymph nodes	19
Lung	9
Pleura	7
Liver	6
Mediastinum	4
Skin	4
Bone	4
Other	1
Mean No. of metastatic sites: 1.8	
Previous treatment	
Radical surgery	12
Palliative surgery	2
Radical radiation therapy	2
Palliative radiation therapy	4
No treatment	31
Performance status (modified Karnofsky scale)	
0	0
1	12
2	30
3	10
4	0
Serum albumin (g/dl)	
> 3.2	35
< 3.2	17

eligible for assessment. Objective responses were seen in 14 (27%) patients (table 2). One patient with pulmonary metastases achieved a complete response lasting for > 8 months. An additional 12 (23%) patients achieved disease stabilization for at least 3 months. Responding patients received a median of eight (range, four to 15) treatment cycles; nonresponding patients received a median of three (range, two to five) treatment cycles. The major pretreatment prognostic factor was found to be performance status (table 2). Objective response or disease stabilization occurred in 25 of 41 patients with a performance status of 1 or 2, while there was only one instance of disease stabilization of ten patients with a performance status of 3. These differences were statistically significant ($x^2 = 6.4$; $P < 0.05$). Serum albumin was correlated with performance status and thus with treatment outcome. The median duration of survival was found to be significantly longer (32 weeks) in patients who either responded to treatment or achieved disease stabilization compared to patients who failed to respond (5 weeks) ($x^2 = 31.6$; $P < 0.05$). The median duration of response to treatment was 26 weeks.

Toxicity from treatment was generally mild. During the initial 4-week period of treatment, leukopenia (wbc count < 3000/mm^3) was seen in 14 (27%) patients. One patient died following an aspiration pneumonia associated with leukopenia of < 1000 cells/mm^3. Depression of the platelet count was less frequent and only three patients showed a fall to between 50,000 and 100,000/mm^3. Thrombocytosis > 800,000 cells/mm^3 was observed in two patients. Neurologic toxic effects consisting of loss of deep-tendon reflexes and/or jaw pain were seen in 26 patients. For the most part neurotoxic effects were mild and transient. Four patients developed moderately severe muscle weakness but it did not require discontinuation of treatment and only one patient developed autonomic neuropathy.

DISCUSSION

The usual approach to treatment of advanced esophageal cancer has been the use of radiation therapy. Although radiation therapy has appeared to offer good results in some of the reported series (6,7), response can only be expected in patients with locoregional disease, whereas most patients in this study had extensive meta-

TABLE 2.—Response to treatment*

	CR	PR	SD	PD	Totals
Sex					
Male	1	7	9	17	34
Female	0	6	3	8	17
Totals	1	13	12	25	51
Performance status					
1	1	5	3	3	12
2	0	8	8	13	29
3	0	0	1	9	10
Totals	1	13	12	25	51
Serum albumin (g/L)					
> 32	1	11	11	11	34
< 32	0	2	1	14	17
Totals	1	13	12	25	51

* CR = complete response, PR = partial response, SD = stable disease, and PD = progressive disease.

static involvement, making chemotherapy the most appropriate choice of treatment. And although it has sometimes been assumed that esophageal cancer behaves in a similar fashion to squamous cell carcinoma of the head and neck (8), this assumption has not been tested. Data from our own institution show response rates of approximately 10% for bleomycin and/or methotrexate,[3] agents which give higher response rates in head and neck cancer. No survival benefit was seen from the administration of either of these agents. In this investigation, vindesine showed definite but limited activity against esophageal cancer, which confirms the reports from other phase II trials in which limited numbers of patients with esophageal carcinoma were treated (9). Response to treatment was associated with symptomatic benefit and prolongation of survival, data which have been infrequently reported in the drug treatment of esophageal cancer. As a single agent, vindesine appears to offer response rates which are at least comparable to those achieved by other drugs and are similar to those achieved by radiation therapy for patients with disease of comparable extent (10).

In view of the activity of this drug in advanced esophageal cancer, studies of combination chemotherapy appear warranted.

[3]Bezwoda WR, and de Moor NG. Unpublished observations. Data of the Oncology Clinic of the Johannesburg Hospital.

REFERENCES

1. TANCINI G, BAJETTA E, and BONADONNA G. Terapia con bleomycin da sola o in associasione con methotrexate nel carcinoma epidermoide dell' esofago. Tumori 60:65-71, 1974.
2. RAWRY MJR, MOORE M, and the SOUTHEASTERN CANCER STUDY GROUP. A phase II pilot study of *cis* platinum (II) in advanced squamous cell esophageal cancer. Proc Am Assoc Cancer Res and ASCO 2:353, 1980.
3. LAD TE. Platinum, mitomycin C and bleomycin chemotherapy of esophageal carcinoma. Proc Am Assoc Cancer Res and ASCO 21:419, 1980.
4. BEDIKIAN AY, VALDIVIESO M, BODEY GP, ET AL. Phase II evaluation of vindesine in the treatment of colorectal and esophageal tumours. Cancer Chemother Pharmacol 2:263-266, 1979.
5. GRALLA RJ, TAN CTC, and YOUNG CW. Vindesine. A review of phase II trials. Cancer Chemother Pharmacol 2:271-274, 1979.
6. PEARSON JG. The value of radiotherapy in the management of esophageal cancer. AJR 105:500-513, 1969.
7. BEATTY JD, DE BOER G, and RIDER WD. Carcinoma of the esophagus: pretreatment assessment, correlation of radiation treatment parameters with survival, and identification and management of radiation treatment failure. Cancer 43:2254-2267, 1979.
8. VOGL SE, GREENWALD E, and KAPLAN BH. Effective chemotherapy for esophageal cancer with methotrexate, bleomycin and cis-diamine dichloroplatinum II. Cancer 48:2555-2558, 1981.
9. KELSEN DP, BAINS M, CVITKOVIC E, ET AL. Vindesine in the treatment of esophageal carcinoma: a phase II study. Cancer Treat Rep 63:2019-2021, 1979.
10. EARLE JD, GELBER RD, MOERTEL CG, ET AL. A controlled evaluation of combined radiation and bleomycin therapy for squamous cell carcinoma of the esophagus. Int J Radiat Oncol Biol Phys 6:821-826, 1980.

Good Response of a Paraneoplastic Neuromyopathy to Cyclophosphamide[1]

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The case history of a 59-year-old man with neuromyopathy and sicca syndrome, preceding the discovery of a small cell bronchial carcinoma for 16 months, is reported. This paraneoplastic neuromyopathy, resistant to steroid therapy, dramatically improved with cyclophosphamide treatment. [Cancer Treat Rep 68:787–789, 1984]

A number of disorders affecting the nervous system or the muscles have been associated with malignancy. One of the most frequent, but also poorly defined, syndromes is the neuromuscular syndrome, also called neuromyopathy, which is mainly associated with bronchial or breast carcinoma. In contrast to the probably high incidence of this syndrome in cancer patients, little is known about its symptomatic treatment. In this discussion, the most important characteristics of this paraneoplastic syndrome, the difficulties in the differential diagnosis, and the possibilities for treatment are reviewed.

CASE REPORT

A 59-year-old man was admitted to the hospital because he had experienced asthenia and slight weight loss for 3 months. In the same period, he noticed a progressive atrophy of the shoulder and pelvic girdle muscles. He simultaneously developed a sicca syndrome with xerostomia, xerophthalmia, and dryness of the skin. His past medical history revealed a recurrent anterior uveitis.

Physical examination showed a man in good general condition, with a marked weakness and amyotrophy of the girdle muscles. The distal muscle strength was completely normal. There were no fasciculations. No exhaustion or facilitation phenomena were observed. Myotatic reflexes were not depressed. A facial erythema was also present. The ophthalmological examination showed a bilateral cataract more pronounced at the right side, which was attributed to the recurrent anterior uveitis. The Shirmer test gave a value of 2 mm within 5 minutes for the right eye and 3 mm for the left eye (normal > 15 mm).

Erythrocyte sedimentation rate, hematological examination, and renal and liver function tests were normal. Creatine kinase activity was normal. Creatinuria was 321 mg/24 hours and creatininuria was 1108 mg/24 hours. Nuclear antigens, ie, antibodies, antibodies against striated muscles, and rheumatoid factor, were not detected. Immunoelectrophoresis of the serum showed an elevated IgA (745 mg/100 ml).

The following investigations were normal: chest roentgenogram, ECG, bone scintigram, laparoscopy with liver biopsy, cytology of the bronchial aspiration, and x-ray studies of the gastrointestinal tract and the renal system. A bronchofibroscopy was also normal and was repeated every 2 months during the further follow-up.

A biopsy of a sublingual salivary gland showed perivascular and interstitial infiltration, while the sialography demonstrated abnormal dilatations of the distal portions of the second- and third-order ductus.

The electromyography showed motor unit potentials of low amplitude and short duration. An interference pattern appeared at a very slight contraction. There were no fibrillations or fasciculations. Repetitive nerve stimulation provoked compound muscle action potentials without decrement. Two muscle biopsies (deltoid and quadriceps) revealed selective atrophy of type II fibers. Necrosis, perivascular or endomysial infiltration, or perifascicular atrophy was not observed.

The differential diagnosis between a neuromuscular syndrome associated with an occult malignancy or a polymyositis in combination with a sicca syndrome was established. A treatment with 40 mg of prednisone/day orally was started. A few weeks later, the sicca syndrome had regressed, but the muscle weakness had worsened, making the patient completely bedridden. At that time, the erythrocyte sedimentation rate was elevated to 45

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mm/hour, while creatine kinase activity was still normal. Prednisone therapy was increased to 60 mg/day, without success.

Ten months after the patient's first complaints, oral cyclophosphamide, at a dose of 2 mg/kg/day, was added to the treatment. Within 4 weeks, a complete remission of the neuromuscular syndrome occurred and the patient was able to resume his normal professional activities. Five months later, he developed a herpes zoster infection of the left shoulder and arm. In view of the possibility of a generalization of the herpetic infection, cyclophosphamide was withdrawn for a few weeks and prednisone was decreased to 20 mg/day. Sixteen months after his initial symptoms, the patient developed a pneumonia of the right upper lobe. A bronchofibroscopy showed a small cell carcinoma with a diameter of 5 mm, localized in the right upper lobe's bronchus. A bone marrow aspiration revealed tumor infiltration.

Despite an intensive chemotherapeutic program consisting of consecutive cycles of vinblastine, doxorubicin, and cyclophosphamide, followed by cisplatin with etoposide, the tumor progressed very rapidly. The patient died 20 months after his initial neuromuscular symptoms. During the 4 months of tumor progression, the neuromyopathy did not recur and the patient stayed ambulatory till some days before death.

At the autopsy, a small cell carcinoma of the right upper lung with metastases in lungs, lymph nodes, liver, and in the base of the skull was found. There were no metastases in the brain and there was no cerebellar atrophy. A few tumor cells at one place in the lumen of an epineural blood vessel were found. The sciatic and sural nerves were normal.

DISCUSSION

The paraneoplastic neuromuscular syndrome or neuromyopathy is a poorly defined entity, characterized by the subacute development of a proximal, symmetrical muscular weakness, mainly associated with wasting and hyporeflexia or areflexia. Sensory deficit is frequently absent or very mild. Defined in this way, as done by most authors (1,2), this syndrome can sometimes be distinguished only with great difficulty from other syndromes giving a similar clinical picture in cancer patients (table 1). The limits between these syndromes are not so clear-cut as could be suggested by their denominations. The diagnostic problems are still more enhanced by the fact that the patient is complaining at a time when the cancer is not detectable or not detected (1,3).

In a prospective study (4), 16 of 30 patients with a proven bronchial carcinoma had a demonstrable weakness affecting predominantly the proximal muscles. Electromyographic studies demonstrated "myopathic" changes together with features of denervation in the same proportion.

TABLE 1.—Syndromes described in cancer patients with the following clinical signs: weakness and wasting of proximal and axial muscles; normal or diminished deep-tendon reflexes; no or mild sensory deficit

Syndrome	Ref No.
Neuromuscular syndrome or neuromyopathy	2,5
Type II muscle fiber atrophy	6
Remote effect of malignancy on intramuscular nerve twigs	7
Carcinoid myopathy	8
Myasthenic syndrome (Lambert-Eaton)	5
Invasion of spinal roots, nerves	9
Ectopic ACTH production, hypercalcemia, hyponatremia, thyreotoxicosis	5
Polymyositis	2
Pure motor neuropathy	10
Subacute motor neuropathy in lymphoma	11
Subacute poliomyelitis in Hodgkin's disease	12
Guillain-Barré syndrome	13

In contrast to the seemingly quite high incidence of this syndrome, remarkably little information is available concerning its treatment. Henson (5) concluded that no specific treatment existed and that the prognosis was determined by the nature of the associated malignant process and its susceptibility to treatment. Spontaneous remissions can occur. Only one author reported on the influence of chemotherapy on the neuromyopathy. Indeed, in 1963, a good response with mechlorethamine therapy was described (14) in a case of neuromuscular syndrome occurring in a 64-year-old man with a bronchial adenoma of the carcinoid type. As in our case, there was no recurrence of the muscular syndrome, despite progression of the tumor.

The treatment of the paraneoplastic neuromuscular phenomena should be twofold. First, all the procedures to control the associated malignancy should be undertaken, eg, surgery, radiotherapy, chemotherapy, and immunotherapy, or a combination of these. On the other hand, a symptomatic treatment should be initiated with the purpose of interfering with the mechanisms that produce the paraneoplastic syndrome. The good response of a carcinoid myopathy (8) to cyproheptadine, a potent serotonin and histamine antagonist, is a good example of the latter. It is impossible to tell why cyclophosphamide was so effective in our patient. Was it by controlling the underlying undetected malignancy or by an immunosuppressive action? Immunological processes can indeed play a role in these syndromes associated with cancer, as demonstrated for the sensory neuropathy described by Brown (*see* ref 15) and for the myasthenic syndrome of Lambert-Eaton (16). In fact, cyclophosphamide was given to our patient as an immunosuppressant in conjunction with steroid therapy for a supposed dermatomyositis with a sicca syndrome. Once the malignancy was detected, the latter diagnosis had to be revised. Indeed, if one considers the diagnostic criteria for dermatomyositis (17), only one of the five criteria was completely fulfilled, the other criteria being partly or not at all fulfilled.

The importance of finding specific criteria to diagnose a paraneoplastic neuromyopathy before detection of the malignancy is once more illustrated. Greater awareness of this disabling paraneoplastic phenomenon is certainly necessary, because within the spectrum of these paraneoplastic syndromes, cyclophosphamide or perhaps other cytostatic agents can be very helpful, as illustrated in our case. Why the neuromyopathy did not recur, either in our patient or in another reported case (14), despite the further progression of the malignancy, remains an enigma and raises questions about its exact relationship with the malignancy.

REFERENCES

1. SHY GM, and SILVERSTEIN I. A study of the effects upon the motor unit by remote malignancy. Brain 88:515–528, 1965.
2. HILDEBRAND J. Lesions of the Nervous System in Cancer Patients. New York, Raven Press, 1978, p 136.
3. SHY GM. The late onset myopathy. World Neurol 3:149–159, 1962.
4. CAMPBELL MJ, and PATY DW. Carcinomatous neuromyopathy: 1. Electrophysiological studies. J Neurol Neurosurg Psychiatry 37:131–141, 1974.
5. HENSON RA. Neuromuscular disorders associated with malignant disease. *In* Disorders of Voluntary Muscle (Walton JN, ed). Churchill Livingstone, 1981, pp 712–724.
6. WARMOLTS JR, PETER NC, RE K, ET AL. Type II muscle fiber atrophy (II-atrophy): an early systemic effect of cancer. Neurology 25:374, 1975.
7. HEFFNER RR, and BARRON SA. Remote effect of malignancy on intramuscular nerve twigs. J Neuropathol Exp Neurol 36:605, 1977.
8. SWASH M, FOW KP, and DAVIDSON AR. Carcinoid myopathy. Arch Neurol 12:572–574, 1975.
9. OLSON MA, CHERNIK NL, and POSNER JB. Infiltration of the leptomeninges by systemic cancer. Arch Neurol 30:122–136, 1974.
10. BRUYN GW. Carcinomatous polyneuropathy. *In* Handbook of Clinical Neurology. Neurological Manifestations of Systemic Diseases. Part 1, 1979, vol 38, pp 679–693.
11. SCHOLD SC, CHO E-S, SOMASUNDARAM M, ET AL. Subacute motor neuronopathy: a remote effect of lymphoma. Ann Neurol 5:271–287, 1979.
12. WALTON JN, TOMLINSON BE, and PEARCE GW. Subacute poliomyelitis and Hodgkin's disease. J Neurol Sci 6:435–445.
13. KLINGON GH. The Guillain-Barré syndrome associated with cancer. Cancer 18:157–1963, 1965.
14. ROWLAND LP, and SCHECK SA. Neuromuscular disorders associated with malignant neoplastic disease. J Chronic Dis 16:777–795, 1963.
15. WILKINSON PC, and ZEROMSKI J. Immunofluorescent detection of antibodies against neurones in sensory carcinomatous neuropathy. Brain 88:559–538, 1965.
16. LANG B, NEWSON DAVIS J, WRAY D, ET AL. Autoimmune etiology for myasthenic (Eaton-Lambert) syndrome. Lancet 2:224–226, 1981.
17. BOHAN A, and PETER JB. Polymyositis and dermatomyositis. N Engl J Med 292:344–347, 403–407, 1975.

Treatment of Recurrent Small Cell Lung Carcinoma With Vindesine and Cisplatin[1,2,3]

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Thirty-two males with recurrent small cell lung cancer after previous remission were treated with vindesine (3–4 mg/m²) plus cisplatin (60–100 mg/m²). Six patients (19%) responded to this therapy with two complete (CR) and four partial remissions. Minor responses were seen in another ten patients (32%). In patients with CR survival from start of treatment lasted 61 and 38 weeks; in patients who did not achieve CR median survival was 12 weeks. Nausea and vomiting were the predominant side effects, while only mild to moderate myelosuppression was noted. The vindesine and cisplatin regimen demonstrated significant activity against heavily pretreated small cell lung cancer, although chemotherapeutic response was poor in regions of prior irradiation. [Cancer Treat Rep 68:791–792, 1984]

The prognosis of small cell lung cancer has been significantly improved by the use of various chemotherapeutic regimens. Remission rates can be achieved in 80%–90% of patients, but long-term survival remains poor, since most patients relapse after 1–2 years (1–3). The current study was undertaken to evaluate the combination of vindesine (VDS) and cisplatin (DDP) in patients with progressive disease following remission.

PATIENTS AND METHODS

Thirty-two previously treated males with histologically confirmed small cell carcinoma of the lung were included in the study at the time of disease progression (table 1). Mandatory preconditions were as follows: no treatment for a minimum of 3 weeks, normal renal and hepatic functions, and full recovery from toxic effects of prior therapy. Five to 30 months (median, 12) had elapsed since initiation of primary treatment. Twenty patients were pretreated with two cytostatic combinations and 12 were treated with only one such combination plus single-agent chemotherapy (table 1). In addition, 25 patients had received primary disease site and mediastinal irradiation. Pretherapeutic evaluations included physical examination; blood chemistry; chest x-rays; iliac crest biopsy; and bone, liver, and brain scans. Additional sonograms and computerized tomograms were done as indicated. Performance status, measurability of disease, and tumor response were classified according to standard criteria (4).

VDS was given at a dose of 3–4 mg/m² by bolus injection on Day 1 followed by 3 L of physiological saline or 5% dextrose during the next 24 hours. DDP was administered at a dose of 60–100 mg/m² as a 6-hour infusion (1000 ml of 0.9% NaCl + 250 ml of 20% mannitol) on Day 2. Chemotherapy was repeated on a 3-week cycle until there was evidence of disease progression or complete remission (CR). In cases of impaired renal function (creatinine levels > 1.5 mg/dl), long-lasting myelosuppression (leukocyte counts < 3000/mm³, platelet counts < 120,000/mm³), or polyneuropathy, the subsequent cycle was postponed for 4–7 days, or alternative regimens were applied. Twenty-nine patients received two to five courses (median, two) of chemotherapy; three received only one course. Eleven patients were started on the lower DDP dosage. Depending on tolerance, doses were increased in the subsequent cycle. One patient was additionally irradiated because of dysphagia due to mediastinal tumor growth.

RESULTS

All 32 patients were evaluable for response. The remission rate was 19%, with two patients (6%) achieving CR and four (13%) achieving partial remission (PR). Minor responses were seen in another ten patients (32%). Re-

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TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Male/female	32/0
Median age in yrs (range): 56 (40-77)	
Median performance status (range): 60% (30%-90%)	
Relapse after—	
Partial remission	12 (38)
Complete remission	20 (62)
Prior chemotherapy	
Doxorubicin, cyclophosphamide, and vincristine	29 (91)
Lomustine, etoposide, and methotrexate	23 (72)
Etoposide	9 (28)
Ifosfamide	3 (9)
Prior radiotherapy *	25 (78)
Stage of disease	
Limited	12 (38)
Extensive	20 (62)
Site of progression	
Lung	27 (84)
Liver	10 (31)
Lymph nodes	10 (31)
Brain	4 (12)
Skeletal system	3 (9)

* Median, 36 Gy (range, 30-36).

sponse rates were correlated to drug dosage. Sites of measurable response included lung, liver, and lymph nodes, while chemotherapeutic response was rare in regions of prior irradiation. CR lasted 34 and 14 weeks (survival, 61 and 38), and PR lasted 22, 14, 14, and 14 weeks (survival, 15-32). Ten additional patients showed minor responses, with a median survival of 15 weeks (range, 12-30). Temporary disease stabilization was induced in ten patients who had disease progression upon entry in the study. The median survival was 12 weeks (range, 8-24). Four of six patients with progressive disease died within 3-5 weeks, and two were changed to other cytostatic schedules.

Toxic effects usually included myelosuppression, nausea, vomiting, and alopecia. Nausea and vomiting occurred in all patients and generally lasted 2-10 days. Leukocyte (median, 2500 cells/mm^3) and platelet (median, 114,000 cells/mm^3) count nadirs were manifested between Days 8 and 14 of each cycle. In 26 cases, recovery to normal values was noticed on Day 22. Persistent nephrotoxicity did not develop. Neurotoxic side effects did not become apparent, probably due to the relatively low VDS doses.

DISCUSSION

In the present trial, 32 males with small cell lung cancer relapsing after remission were treated with a combination of VDS and DDP. The study was based on the encouraging remission rates of 15%-30% noted for DDP as a single agent in pretreated small cell lung cancer (5), although, more recently, other investigators have reported lower response rates in similar situations (6). A response rate of about 15% (range, 7%-27%) has been demonstrated using single-agent VDS as second- or third-line therapy in small cell lung cancer (7,8). Prior treatment with vincristine had a small effect on response to VDS. According to these data, a dose-dependent lack of cross-resistance could be demonstrated in vitro using vincristine-resistant lines of murine lymphoblasts (9). Moreover, the finding of increased activity of VDS in cells selected for resistance against DDP serves as an experimental base for the VDS and DDP combination (10).

Our study has shown that the VDS and DDP combination is effective in patients with extensively pretreated small cell lung cancer. At a minimum, temporary reductions of even widespread hepatic, lymphatic, and skeletal metastases could be achieved. However, response in previously irradiated intrathoracic areas, the most frequent location of relapse (3), was poor. In addition to extensive chemical pretreatment, this observation may account for the relatively low remission rate of 19% and the short median survival of 14 weeks.

REFERENCES

1. LIVINGSTON RB, MOORE TN, HEILBRUN L, ET AL. Small-cell carcinoma of the lung: combined chemotherapy and radiation. A Southwest Oncology Group study. Ann Intern Med 88:194-199, 1978.
2. AISNER J, WHITACRE M, VAN ECHO DA, ET AL. Combination chemotherapy for small cell carcinoma of the lung: continuous versus alternating non-cross-resistant combinations. Cancer Treat Rep 66: 221-230, 1982.
3. NIEDERLE N, KRISCHKE W, SCHULZ U, ET AL. Untersuchungen zur kurzzeitigen Induktions- und zyklischen Erhaltungstherapie beim inoperablen kleinzelligen Bronchialkarzinom. Klin Wochenschr 60:829-838, 1982.
4. MILLER AB, HOOGSTRATEN B, STAQUET M, ET AL. Reporting results of cancer treatment. Cancer 47:207-214, 1981.
5. CAVALLI F, GOLDHIRSCH A, SIEGENTHALER P, ET AL. Phase-II study with *cis*-dichlorodiammineplatinum(II) in small cell anaplastic bronchogenic carcinoma. Eur J Cancer 16:617-621, 1980.
6. BHUCHAR VK, and LANZOTTI VJ. High-dose cisplatin for lung cancer. Cancer Treat Rep 66:375-376, 1982.
7. NATALE RB, GRALLA RJ, and WITTES RE. Phase II trial of vindesine in patients with small cell lung carcinoma. Cancer Treat Rep 65:129-131, 1981.
8. ØSTERLIND K, DOMBERNOWSKY P, SØRENSEN PG, ET AL. Vindesine in the treatment of small cell anaplastic bronchogenic carcinoma. Cancer Treat Rep 65:245-248, 1981.
9. HILL BT, and WHELAN RDH. Establishment of vincristine-resistant and vindesine-resistant lines of murine lymphoblasts in vitro and characterisation of their patterns of cross-resistance and drug sensitivities. Cancer Chemother Pharmacol 8:163-169, 1982.
10. SEEBER S, OSIEKA R, SCHMIDT CG, ET AL. In vivo resistance towards anthracyclines, etoposide, and *cis*-diamminedichloroplatinum(II). Cancer Res 42:4719-4725, 1982.

Randomized Phase II Trial of Hexamethylmelamine Versus Pentamethylmelamine in Carcinoma of the Bilharzial Bladder[1,2]

Nazli Gad-el-Mawla,[3] John L. Ziegler,[4,*] Reda Hamza,[3] Moustafa Elserafi,[3] and Hussein Khaled[3]

In 1979, we reported the results of hexamethylmelamine (HMM) treatment in patients with carcinoma of the bilharzial bladder. Of 26 evaluable patients receiving 300 mg/m²/day, there were ten partial responses (1). The major toxic effect of this agent was nausea and vomiting, which was experienced in varying degrees by all patients. This side effect forced discontinuation of the drug in some patients, and undoubtedly influenced drug compliance adversely. The recent availability of the HMM analog pentamethylmelamine (PMM), a water-soluble and thus injectable compound, prompted a comparative phase II clinical trial in an effort to improve the therapeutic index of the melamines in patients with this tumor (2).

PATIENTS AND METHODS

Patients admitted to this trial had histologically and cytologically confirmed carcinoma of the bilharzial bladder and were treated at the National Cancer Institute, Cairo, Egypt. All patients had measurable tumor and were staged clinically and radiographically, according to International Union Against Cancer criteria. Consenting patients with inoperable T_3 or T_4 bladder tumors whose creatinine clearance was > 60 ml/minute were eligible for treatment.

Pretreatment evaluation consisted of bimanual pelvic examination, cystoscopy, and iv pyelogram, in addition to routine cbcs, electrolytes, BUN, and liver function tests.

Following evaluation, patients were stratified according to histologic type (squamous cell vs other) and stage (T_3 vs T_4) and were randomized (by table of random numbers) to receive HMM at a dose of 300 mg/m²/day orally or PMM at a dose of 750 mg/m²/day iv (in a 6-hour 500-ml isotonic saline infusion) for 5 days, every 3 weeks. The dose and schedule of HMM were identical to those in our previous study (1). The dose and schedule of PMM were selected from preliminary phase I trial results (2-7) because of pharmacologic, toxicologic, cell kinetic, and administrative considerations. Patients receiving < 60 days of HMM or four courses of PMM were considered inevaluable. Patients were hospitalized for at least the first 3 weeks of therapy to assure drug compliance and to evaluate nausea and vomiting. Patients were evaluated clinically every 2 weeks by bimanual pelvic examination, supplemented by iv pyelography or pelvic ultrasonography, if indicated. Tumor dimensions are easily measured by these methods. Criteria for response are the same as those reported previously from this institution (1). Complete response (CR) was defined as the disappearance of all measurable tumor; partial response (PR) was defined as a reduction of the product of cross diameters of the tumor by 50%; disease improvement was defined as an objective response but less than a PR; and stable disease was defined as no increase in tumor size during therapy.

RESULTS

Sixty patients were entered in the trial: 15 were inevaluable because of failure to return to the clinic. We were unable to ascertain the reasons for default. Of the 45 evaluable patients, 20 received HMM and 25 received PMM. Responses are shown in table 1. Of 20 patients receiving HMM, seven achieved PR (35%); an additional

TABLE 1.—Responses to HMM and PMM in a randomized clinical trial

	No. of evaluable patients	No. of patients with—			
		CR	PR	Disease improvement/ stable disease	No response
HMM	20	0	7*	8	5
PMM	25	1†	7†	11	6

*Squamous cell carcinoma, 5 patients; transitional cell carcinoma, 1 patient; and adenocarcinoma, 1 patient.

†Squamous cell carcinoma, 5 patients; and transitional cell carcinoma, 3 patients.

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[2]Supported by Public Health Service grant CA-30348 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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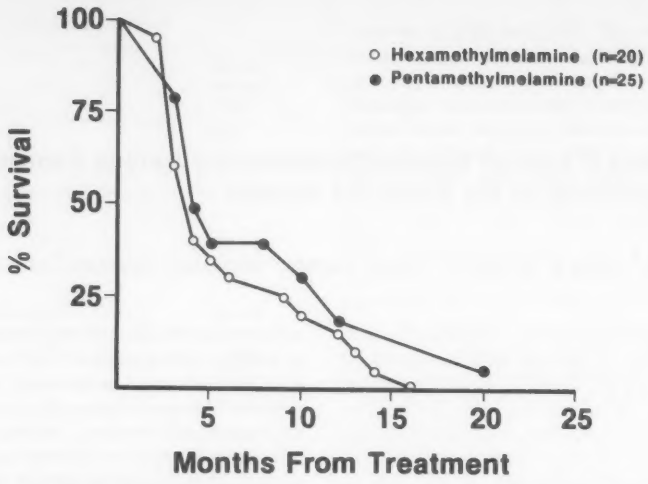

FIGURE 1.—Survival in 45 patients receiving hexamethylmelamine (HMM) (n = 20; open circles) or pentamethylmelamine (PMM) (n = 25; solid circles). There is no difference in outcome in these 2 groups.

eight patients (40%) had either disease improvement or stable disease during treatment. Responses lasted 6-20+ months (median, 12). Of 25 patients receiving PMM, one CR and seven PRs were observed (32%); an additional 11 patients (44%) had disease improvement or stable disease. Responses lasted 4-18+ months (median, 11) and include two patients who are alive without disease at 6+ months following surgical resection of residual tumor (ie, radical cystectomy). Survival in the two groups is identical (fig 1).

Toxicity of both treatment groups was equivalent and consisted largely of moderate nausea and vomiting (HMM, 19 of 20 patients; PMM, 22 of 25). As was similar to our previous trial, patients receiving HMM developed nausea and vomiting during the initial 2-3 weeks of treatment. These symptoms tapered in severity with continued treatment and were improved temporarily by phenothiazine administration. Patients receiving PMM were nauseous only during the infusion period. No other significant side effects were noted. Neurotoxicity cited in phase I trials was not encountered (2-7). Liver function tests were not abnormal. Mild leukopenia was encountered in two patients on each treatment arm. Deterioration in renal function (increase in serum creatinine) was observed in three of 25 patients receiving PMM. Whether this was due to the drug itself or to the associated bilharziasis is not clear.

DISCUSSION

This trial demonstrates similar clinical activities of HMM and PMM in carcinoma of the bilharzial bladder with respect to response rates, remission durations, and survival. Both compounds produced moderate nausea and vomiting. The advantages of PMM over HMM in the Egyptian setting are the limited duration of nausea and vomiting and the certainty of drug compliance. PMM is now under consideration for use in combination with other active agents.

REFERENCES

1. GAD-EL-MAWLA NM, MUGGIA FM, HAMZA MR, ET AL. Chemotherapeutic management of carcinoma of the bilharzial bladder: a phase II trial with hexamethylmelamine and VM-26. Cancer Treat Rep 62:993-996, 1978.
2. EAGAN RT, CREAGAN ET, RUBIN J, ET AL. Early clinical trial of a 1-day intermittent schedule for pentamethylmelamine. Cancer Treat Rep 64:993-995, 1980.
3. GOLDBERG RS, GRIFFIN JP, MCSHERRY JW, ET AL. Phase I study of pentamethylmelamine. Cancer Treat Rep 64:1319-1322, 1980.
4. VAN ECHO DA, CHIUTEN DF, WHITACRE M, ET AL. Phase I trial of pentamethylmelamine in patients with previously treated malignancies. Cancer Treat Rep 64:1335-1339, 1980.
5. IHDE DC, DUTCHER JS, YOUNG RC, ET AL. Phase I trial of pentamethylmelamine: a clinical and pharmacologic study. Cancer Treat Rep 65:755-762, 1981.
6. CASPER ES, GRALLA RJ, LYNCH GR, ET AL. Phase I and pharmacological studies of pentamethylmelamine administered by 24-hour intravenous infusion. Cancer Res 21:1402-1406, 1981.
7. AJARI JA, CABANILLAS FF, and BODEY GP. Phase I trial of pentamethylmelamine. Cancer Treat Rep 66:1227-1228, 1982.

Phase II Trial of Prednimustine in Glioblastoma Multiforme[1,2]

Jørn Andersen,[3,*] Leif Christensen,[3] and H. Kongsholm[4]

Twenty-one consecutive patients with histologically confirmed glioblastoma multiforme were treated with prednimustine (130 mg/m² for 5 days every 3 weeks) adjuvant to surgery and radiation therapy. A median survival of 8.7 months was obtained, and prednimustine seems to be of limited effectiveness. Toxicity was acceptable and primarily hematologic. [Cancer Treat Rep 68:795–797, 1984]

Glioblastoma multiforme (GM) is the most malignant of the primary brain tumors, and the course of the disease is invariably fatal. At present, most patients with GM are treated with surgical resection followed by radiotherapy, since this seems to significantly increase survival (1,2). Numerous trials have been performed evaluating adjuvant chemotherapy, primarily concentrating on the nitrosoureas (since these seem to cross the blood-brain barrier). So far, the results have been disappointing with little or no increase in survival, which seems to converge with almost no patients alive 2 years after surgery (1–4).

Prednimustine, a chlorambucil ester of prednisolone, has shown an improved therapeutic index compared to prednisolone and chlorambucil given as separate drugs, probably due to increased intracellular uptake and hydrolysis with concomitant high intracellular concentrations of the cytotoxic compound (5,6). The drug has been found active in the treatment of lymphoproliferative disorders (7), as well as in the treatment of solid tumors (8–10). Prednimustine is lipid-soluble and thus, like the nitrosoureas, should be able to cross the blood-brain barrier. The dose chosen was 130 mg/m² given for 5 days every 3 weeks, which is comparable to that given in other studies where the hematologic toxicity was rather pronounced (7,10,11). The drug is given orally and has relatively few subjective side effects, which makes it well-suitable for use on an outpatient basis. On the basis of these criteria, a phase II trial of prednimustine in the treatment of patients with GM was performed.

PATIENTS AND METHODS

Twenty-one consecutive patients with GM were entered in the study. Criteria for inclusion were as follows: histologically confirmed GM, ⩽ 65 years of age, evaluable disease parameters, wbc count ⩾ 3.0×10^9/L, and informed consent. Criteria for exclusion were as follows: other malignant disease, serious chronic infection, diabetes mellitus, or a record of peptic ulcer. The patient characteristics are shown in table 1. After surgery, which was performed as radical as possible, the whole brain was irradiated with 4-MeV photons through parallel opposed fields to a dose of 36 Gy/18 fractions, followed by a tumor boost dose of 18 Gy/9 fractions. Chemotherapy, as previously mentioned, was started simultaneously with radiotherapy and the dose was adjusted according to hematologic toxicity: wbc count ⩾ 3.0×10^9/L and platelet count ⩾ 100×10^9/L: 100% dose; wbc count 2.0–2.9 $\times 10^9$/L and platelet count 75–99 $\times 10^9$/L: 50% dose; wbc count 1.0–1.9 $\times 10^9$/L and platelet count 50–74 $\times 10^9$/L: 25% dose; and wbc count < 1.0×10^9/L and platelet count < 50×10^9/L: 0% dose. Treatment was stopped at tumor recurrence or at the end of 3 years. All patients received postoperative corticosteroid and/or anticonvulsive treatment for varying periods. The patients were evaluated every 3 weeks with clinical examination and blood tests. If the clinical examination showed progression of neurological symptoms, computerized tomographic scan and/or radioisotope scintigraphy were performed to verify tumor recurrence, and chemotherapy was eventually discontinued. Disease progression was defined as an increase in tumor size on computerized tomographic scan or scintigraphy or as progressive neurological deterioration.

RESULTS

As shown in figure 1, the median time from start of ir-

[1]Received June 13, 1983; revised Oct 21, 1983; accepted Dec 19, 1983.

[2]Prednimustine (Leo 1031) tablets (20 and 100 mg) were produced at AB LEO, Helsingborg, Sweden, and were delivered through H. Lundbeck and Co, A/S, Copenhagen, Denmark.

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TABLE 1.—Patient characteristics

Characteristic	
Total No. of patients	21
Sex	
Males	12
Females	9
Median age in yrs (range)	56 (14-65)
Median duration of symptoms in mos (range)	2 (½-36)
No. of patients with—	
Tumor localization	
Frontal	5
Parietal	6
Temporal	7
Occipital	1
Deep	2
Performance status (Karnofsky scale)	
80-100	6
60-70	5
40-50	5
10-30	5
Median—	
Survival in mos (range)	8.7 (1.6-43.4+)
No. of treatment cycles (range)	12 (1-52)
Duration of treatment in mos (range)	8 (1-36)
Leukocyte count nadir* (range)	3.8 (2.2-6.8)
Platelet count nadir* (range)	165 (47-342)

* × 10^9 cells/L.

radiation and chemotherapy to disease progression was 8.2 months; the overall median survival was 8.7 months. As in other studies, there is a small proportion of long-term survivors (three of 21 patients).

Toxicity

Treatment duration and hematologic toxicity are shown in table 1. Toxicity was surprisingly moderate and primarily hematologic. No signs of cumulative hematologic toxicity were observed. Four patients experienced nausea and one developed diabetes (this patient was receiving dexamethasone treatment because of radiation-induced brain edema). No peptic ulcers or psychic disturbances were noted and no treatment-related deaths were observed.

DISCUSSION

After surgery, radiotherapy plays the most significant role in prolonging survival of patients with GM. In most studies, adjuvant chemotherapy has shown only marginal or no effect. Prednimustine seems to be no exception. The median survival in this study is lower than that in other adjuvant studies, but we have included only patients with GM, while most other studies include patients with grade III astrocytomas. Furthermore, this study consists of 21 consecutive patients with no exclusion of

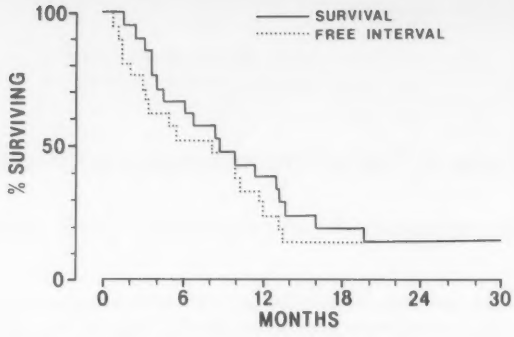

FIGURE 1.—Survival and disease-free interval from start of irradiation and chemotherapy.

patients in relatively poor condition, or who did not receive a minimal amount of chemotherapy. It should also be noted that there are a small number of long-term survivors in this study.

Prednimustine was well-tolerated and showed only few and moderate side effects.

Until now, the results of chemotherapy in the treatment of GM have been disappointing, and new treatment modalities and chemotherapeutic agents must be investigated in the hope of overcoming the overwhelming biology of this devastating disease.

REFERENCES

1. WALKER MD, ALEXANDER E, HUNT E, ET AL. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas: a cooperative clinical trial. J Neurosurg 49:333-343, 1979.
2. WALKER MD, GREEN SB, BYAR DP, ET AL. Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. N Engl J Med 303:1323-1329, 1980.
3. European Organization for Research on Treatment of Cancer (EORTC) Brain Tumor Group. Evalution of CCNU, VM-26 plus CCNU, and procarbazine in supratentorial brain gliomas. J Neurosurg 55:27-31, 1981.
4. GREEN SB, BYAR DP, WALKER MD, ET AL. Comparisons of carmustine, procarbazine, and high-dose methylprednisolone as addition to surgery and radiotherapy for the treatment of malignant glioma. Cancer Treat Rep 67:121-132, 1983.
5. KÖNYVES I, NORDENSKÖLD B, PLYM-FORSHELL G, ET AL. Preliminary clinical and absorption studies with prednimustine in patients with mammary carcinoma. Eur J Cancer 11:841-844, 1975.
6. WILKINSON R, GUNNARSON PO, PLYM-FORSHELL G, ET AL. The hydrolysis of prednimustine by enzymes from normal and tumour tissues. Excerpta Medica 420:260-280,1978.
7. PEDERSEN-BJERGAARD J, MØRK HANSEN M, GEISLER CH, ET AL. Clinical trial of prednimustine, Leo-1031 (NSC-134087), in patients with non-Hodgkins lymphomata and chronic lymphocytic leukemia previously treated with steroids and alkylating agents. Acta Med Scand 207:215-220, 1980.
8. MOURIDSEN HT, KRISTENSEN D, HALSKOV NIELSEN J, ET AL. Phase II trial of prednimustine, L-1031 (NSC-1031087) in advanced breast cancer. Cancer 46:253-255, 1980.
9. CATANE R, KAUFMAN JH, MADAJEWCZ S, ET AL. Prednimustine therapy for advanced prostatic cancer. Br J Urol 50:29-32, 1978.
10. JOHNSSON J-E, TROPÉ C, MATHSSON W, ET AL. Phase II study of Leo

1031 (prednimustine) in advanced ovarian carcinoma. Cancer Treat Rep 63:421-424, 1979.
11. MØLLER T, HÅKONSSON L, KÖNYVES I, ET AL. Prednimustine in the treatment of non-Hodgkin's lymphomas. *In* Proceedings of the International Conference of Malignant Lymphoma, Lugano, Switzerland, 1981, p 48.

Continuous Infusion Versus Intermittent Bolus Injection of Bleomycin in a Human Embryonal Testicular Cancer Xenograft[1,2]

Rainhardt Osieka,* Peter Glatte, and Carl-Gottfried Schmidt[3]

In vivo experiments with bleomycin in conventional murine tumor systems indicate a superiority of continuous-infusion schedules over daily intermittent bolus injections. This schedule of administration has been incorporated into many clinical trials, especially in testicular cancer, on the basis of in vitro and in vivo experiments as well as favorable comparisons with historical controls. Since mechanisms linked to a superiority of continuous-infusion schedules may not be operative in every individual tumor line, we have compared both schedules of administration in a human testicular cancer xenograft. We found no significant difference with respect to antineoplastic response. [Cancer Treat Rep 68:799–801, 1984]

Primarily on the basis of in vivo and in vitro experiments, changes in the schedule of drug administration have been proposed as a way to improve therapeutic index. At the preclinical level, relevant in vivo studies with bleomycin have been performed with tumors of murine origin such as P388 mouse leukemia (1) and Lewis lung carcinoma (2), while at the clinical level, randomized studies comparing both schedules have never been performed.

Heterotransplantation of human cancers into immune-deprived murine hosts has been achieved with great success in many laboratories, and the validity of this tumor model has been demonstrated by correlating preclinical and clinical drug responses (3).

Using osmotic minipumps we have compared continuous ip drug administration of bleomycin with daily intermittent bolus injections of the drug via the same route at various dose levels in a human embryonal testicular cancer xenograft.

MATERIALS AND METHODS

Bleomycin was used as commercially available from H. Mack, Illertissen, W Germany. The drug was dissolved in physiologic saline so that a constant volume of 0.01 ml of this solution/g of body weight could be applied over a total dose range from 10.5 to 42 mg/kg by daily injections for 1 week. For continuous-infusion schedules osmotic minipumps (Alzet Nr. 2001, Alza Corp, Palo Alto, CA) were filled with bleomycin solutions prepared in such concentrations that the total dose to be delivered was contained in 0.17 ml. The pumps were implanted intraperitoneally under light ether anesthesia.

The human tumor xenograft line Ma was established from a surgically resected brain metastasis in a male patient with a chorionic gonadotropin-producing embryonal carcinoma of the testis. Previously, the pulmonary metastases of the donor patient had responded to treatment with bleomycin and vinblastine. Male random-bred (National Institutes of Health-Swiss background), congenitally athymic (nu/nu) mice, 3–6 weeks old, served as recipients for sc inoculation of tumor fragments by trocar and were randomized to treatment groups 3 weeks later. Tumor volumes (V) were calculated from the largest (L) and smallest (B) diameters by the formula

$$V = 0.5 \times L \times B^2$$

and were transformed to relative tumor volumes with all values set to 1 at initiation of treatments. Rather than making statistical comparisons of relative tumor volumes at a single time point, we have calculated areas under the curve for relative tumor diameters for each tumor-bearing animal until Day 15 after initiation of treatment; these values were subjected to multivariate analysis and nonparametric statistical tests (4).

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RESULTS

The production of human chorionic gonadotropin correlated well with tumor volumes, and histology was preserved well in the xenograft situation. In figure 1 mean relative tumor volumes are plotted for intermittent bolus injections versus continuous infusions. Doses of bleomycin > 42 mg/kg were poorly tolerated and are in excess of the LD10/30 value. The 84-mg/kg dose is included in figure 1 to illustrate the dose response, but the data from this dose level were excluded from statistical comparison because too many mice died before Day 15. Multivariate analysis revealed a clear dependency of area under the curve values on dose ($P \leq 0.05$) but not on schedule of administration ($P \geq 0.05$). This was confirmed by nonparametric tests (Kruskal-Wallis). Thus, our data do not support a superiority of continuous infusion over daily intermittent injections in a human testicular cancer xenograft.

DISCUSSION

Cytotoxicity of bleomycin is cell-cycle-dependent and increases with duration of exposure following a very steep dose-response curve (5). Repair of potentially lethal damage has been linked to this phenomenon (6) and is thought to be minimized by continuous-infusion schedules (7). Conflicting results have been reported by other investigators (8,9) who did not observe increased antineoplastic activity after fractionated or continuously infused doses of bleomycin.

Using Lewis lung carcinoma, Sikic et al (2) have demonstrated a superiority of continuously infused bleomycin over intermittent injections of the drug, with increased inhibition of tumor growth and less pulmonary toxicity at 40 mg/kg. Peng et al (1) have compared the cell killing of P388 leukemic spleen colony-forming units with both modes of administration and also found enhanced antileukemic activity with continuous infusion.

Our data do not contradict such findings but rather serve to illustrate that the mechanism whereby continuous-infusion schedules improve the antineoplastic properties of bleomycin may not be operative in every individual human tumor line. An improved therapeutic index for continuously infused bleomycin, however, may still result from reduced pulmonary toxicity (10).

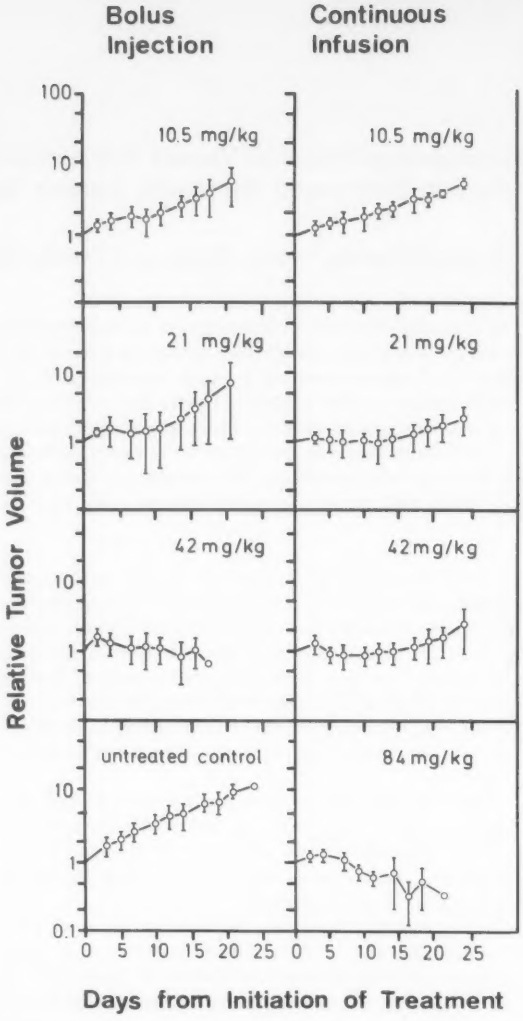

FIGURE 1.—Plot of mean relative tumor volumes ± SD, comparing the efficacy of bleomycin given ip by daily bolus injections or by continuous infusion using osmotic minipumps over 7 days.

REFERENCES

1. PENG YM, ALBERTS DS, CHEN HSG, ET AL. Antitumour activity and plasma kinetics of bleomycin by continuous and intermittent administration. Br J Cancer 41:644-647, 1980.
2. SIKIC BI, COLLINS JM, MIMNAUGH EG, ET AL. Improved therapeutic index of bleomycin when administered by continuous infusion in mice. Cancer Treat Rep 62:2011-2017, 1978.
3. STEEL GG, COURTENAY VD, and PECKHAM MJ. The response to chemotherapy of a variety of human tumour xenografts. Br J Cancer 47:1-13, 1983.
4. LESSER ML, BRAUN HI, and HELSON L. Statistical methods for measuring and comparing treatment efficacies: application to nude mice experimentation. Exp Cell Biol 48:126-137, 1980.
5. DREWINKO B, NOVAK JK, and BARRANCO SC. The response of human lymphoma cells in vitro to bleomycin and 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res 32:1206-1208, 1972.
6. BARRANCO SC, and BOLTON WE. Cell cycle phase recovery from bleomycin-induced potentially lethal damage. Cancer Res 37:2589-2591, 1977.
7. TAKABE Y, MIYAMOTO T, WATANABE M, ET AL. Bleomycin: mammalian cell lethality and cellular basis of optimal schedule. JNCI 59:1251-1255, 1977.
8. SUZUKI Y, URANO M, ANDO K, ET AL. Repair of potentially lethal damage after single injection or continuous infusion of bleomycin.

Gan 69:195-199, 1978.
9. TWENTYMAN PR. Dose fractionation does not prevent repair of potentially lethal damage induced by bleomycin (NSC-125066) in vivo. Cancer Treat Rep 60:259-260, 1976.
10. COOPER KR, and HONG WK. Prospective study of the pulmonary toxicity of continuously infused bleomycin. Cancer Treat Rep 65:419-425, 1981.

Evaluation of Vinblastine Administered by 5-Day Continuous Infusion in Women With Advanced Breast Cancer[1,2]

James N. Ingle,[3] David L. Ahmann,[3] James G. Gerstner,[4] Stephanie J. Green,[3] Michael J. O'Connell,[3] and Larry K. Kvols[3,*]

Yap et al (1) reported the use of vinblastine (VBL) (administered by continuous 5-day infusion at doses of 1.4-2.0 mg/m^2/day every 3 weeks) in 34 patients whose breast cancer was refractory to more conventional agents, including doxorubicin, 5-FU, cyclophosphamide, and methotrexate. In the 30 patients they considered evaluable for response, they found there was a 40% response rate (one complete and 11 partial responses). Because of the need to establish regimens with increased efficacy in women who have failed combination chemotherapy, we judged that corroboration of these results was necessary and conducted the following clinical trial.

PATIENTS AND METHODS

Eligibility requirements included histologic confirmation of breast cancer and progressive metastatic disease. Patients were required to have a wbc count $\geq$ 4100/mm^3, platelet count $\geq$ 130,000/mm^3, creatinine $<$ 1.5 mg/dl, and total bilirubin $\leq$ 1.5 mg/dl. Radiation therapy (concomitant or within the previous month) to nonindicator lesions was permissible if it involved $\leq$ 15% of the marrow. Patients who had $>$ 30% of the marrow irradiated were not eligible. VBL was administered at a dose of 1.6 mg/m^2/day by constant infusion for 5 consecutive days via a central venous catheter. The planned cycle length was 3-4 weeks. The response criteria employed required a $\geq$ 50% reduction in the product of perpendicular diameters of a measurable indicator lesion for a partial regression, and a definite decrease in an evaluable (nonmeasurable) indicator lesion for a regression.

The study was designed to accrue 15 patients with measurable disease initially, and, if any responses were observed, to extend the accrual to a total of 30 patients. Originally, patients with evaluable disease were also allowed entry to the study, but the protocol was modified to accept only patients with measurable disease. Considering measurable patients only, seven or more regressions among 30 patients would have been considered evidence of meaningful activity. Under this design, the probabilities of concluding that VBL was active were 0.84 and 0.03, for true regression probabilities of 0.3 and 0.1, respectively. Thus, the probability of detecting an active regimen (ie, regression probability $\geq$ 0.3) was high, while the probability of falsely declaring activity in an inactive regimen (ie, regression probability $\leq$ 0.1) was low.

RESULTS

Fifteen patients with measurable disease were entered in this study (table 1). No objective regressions were observed among these 15 patients. One patient developed a catheter-related staphylococcal sepsis and received only 68% of the full dosage during the first and only course of therapy. An additional six patients also had disease progression by the time of the first reassessment. All patients but one have died; the median survival was 134 days (range, 14-435). The most common toxic effect was myelosuppression; one patient had leukopenia-associated sepsis. Three patients had received concomitant radiation therapy. One patient required hospitalization for an adynamic ileus, but otherwise, nonhematologic toxicity was generally mild and infrequent. In addition, one patient with evaluable disease was treated and also did not respond.

DISCUSSION

Based on no responses among 15 patients with measurable disease, a 95% confidence interval for the regression probability of patients represented by this study, calculated according to the approach of Jennison and Turnbull (2) for multistage designs, is from 0 to 0.22. Thus, substantial antitumor activity is unlikely in this population.

Two additional studies have been reported examining

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[2]Conducted as a collaborative trial of the Mayo Clinic and the North Central Cancer Treatment Group and supported in part by Public Health Service grant CA-25224 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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TABLE 1.—Clinical data for 15 patients with measurable disease

Characteristic	No. of patients
Age (yrs)	
Median: 55	
Range: 32–70	
Time from diagnosis of metastases to this clinical trial (mos)	
Median: 17.2	
Range: 5.3–75.5	
Eastern Cooperative Oncology Group performance score	
0	1
1	7
2	4
3	3
Dominant disease status	
Soft tissue	1
Osseous	2
Visceral	12
Prior hormonal therapy	14
No. of prior hormonal therapy regimens	
Median: 2	
Mean: 2.4	
Range: 0–5	
Prior chemotherapy regimens	
Cyclophosphamide, 5-FU, and prednisone	12
Cyclophosphamide, methotrexate, and 5-FU	1
Doxorubicin	9
Doxorubicin and vincristine	1
Doxorubicin and cyclophosphamide	1
Cyclophosphamide, doxorubicin, and cisplatin	3
Cyclophosphamide, doxorubicin, methotrexate, and 5-FU	1
5-FU, dibromodulcitol, and prednisone	1
Melphalan	1
Dibromodulcitol	1
No. of prior chemotherapy regimens	
1	1
2	12
3	2
Total No. of prior systemic therapy regimens	
Median: 4	
Mean: 4.3	
Range: 2–7	
No. of VBL treatment cycles	
1	7
2	3
3	4
4	1
Hematologic toxicity (Cycle 1)	
Wbc count nadir (cells/mm^3)	
No. of patients: 15	
Median: 1700	
Range: 500–4400	
Platelet count nadir (cells/mm^3)	
No. of patients: 13	
Median: 243,000	
Range: 106,000–425,000	

the VBL infusion approach with conflicting results (3,4). Although Yap et al (1) reported that responses were seen in all organ sites of involvement, only 13 (43%) of their patients had visceral dominant disease. The proportion of patients with visceral dominant disease in our study (80%) was almost twice as high. It is probable that inherent differences between patient populations examined in the two studies account, to some extent, for the different results obtained. However, we conclude that VBL administered by 5-day continuous infusion was not associated with demonstrable clinical benefit in our patients with advanced breast cancer who had failed prior cytotoxic chemotherapy.

REFERENCES

1. YAP H-Y, BLUMENSCHEIN GR, KEATING MJ, ET AL. Vinblastine given as a continuous 5-day infusion in the treatment of refractory advanced breast cancer. Cancer Treat Rep 64:279–283, 1980.
2. JENNISON C, and TURNBULL BW. Confidence intervals for a binomial parameter following a multistage test with application to MIL-STD 105D and medical trials. Technometrics 25:49–58, 1983.
3. TANNOCK I, ERLICHMAN C, PERRAULT D, ET AL. Failure of 5-day vinblastine infusion in the treatment of patients with advanced refractory breast cancer. Cancer Treat Rep 66:1783–1784, 1982.
4. SHAH MK, CREECH RH, CATALANO RB, ET AL. Phase II study of vinblastine infusion in the treatment of refractory advanced breast cancer. Proc ASCO 1:73, 1982.

Phase I–II Study of High-Dose Mitomycin With Autologous Bone Marrow Transplantation in Refractory Metastatic Breast Cancer[1,2]

Nizar Tannir,* Gary Spitzer, Karel Dicke, Frank Schell, Alfred DiStefano, and George Blumenschein[3,4]

Mitomycin (MMC) alone or in combination with other agents has been one of the effective second-line regimens for the treatment of metastatic breast cancer (1,2). Since in conventional doses its major toxicity has been hematologic (1), we started this pilot study of high-dose MMC and autologous bone marrow transplantation (ABMT) in patients with refractory metastatic breast cancer, with the hope of improving the response rate without undue hematologic toxicity.

PATIENTS AND METHODS

Patients were considered eligible for this study if they met the following criteria: advanced, pathologically defined metastatic breast cancer refractory to standard therapy; absence of bone or bone marrow metastasis at the time of bone marrow harvest; normal hematologic status (wbc count $\geq 4000/\mu l$, platelet count $\geq 150{,}000/\mu l$); and normal hepatic and renal functions (SGOT ≤ 40 mU/ml, serum bilirubin ≤ 1.3 mg/dl, and serum creatinine ≤ 1.4 mg/dl). In the initial phase of the study, eight patients received MMC at a standard dose of 20 mg/m^2 as a single iv bolus without ABMT. Subsequently, in this first group of patients, the dose of MMC was escalated to 30 mg/m^2/course (four patients), 40 mg/m^2/course (three), and 50 mg/m^2/course (one). ABMT was performed 72–96 hours after MMC administration. Because of rapid disease progression in this group of patients and the development of fatal renal toxicity in the patient treated with 50 mg/m^2, we decided that subsequent patients entered in the study would receive, from the start, high-dose MMC at 40 mg/m^2 followed by ABMT. Unless they developed progressive disease after the first course of high-dose MMC, all patients received a second course of high-dose MMC 4–6 weeks after the first course or after recovery from the toxicity of the first course. Standard criteria for response to therapy were utilized.

RESULTS

The overall responses are shown in table 1. All responses were of short duration (< 3 months). MMC, at doses ranging from 30 to 50 mg/m^2/course, produced severe myelosuppression in all patients and irreversible thrombocytopenia in some (table 1). Two patients, each of whom had received a total of 450 mg/m^2 of doxorubicin prior to MMC, developed intractable congestive heart failure which was the cause of death of one. Two other patients developed pneumonitis and died of progressive pulmonary insufficiency. Gastrointestinal toxic effects included diarrhea, ileus, and significant mucositis in 20% of patients after the first course of high-dose MMC and in 40% after the second course. No patient developed hepatic dysfunction secondary to MMC; in four patients who had postmortem examination, no evidence of veno-occlusive disease was found. There was one death due to myelosuppression-related complications with septicemia and disseminated intravascular coagulation.

DISCUSSION

Following the analysis of this study we decided to discontinue the high-dose MMC treatment program because of excessive nonhematologic toxicity and failure to show a convincing evidence of a dose-response relation with MMC. With twofold escalation of MMC, nonhematologic toxicity was more frequent and severe following the second course, suggesting cumulative toxicity. In this study, the nonhematologic toxicity affected different organ systems including the heart, lungs, kidneys, and gastrointestinal tract. However, severe enterocolitis, pancreatitis, or

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[4]We thank Ms. Dean Cooke for her secretarial expertise in the preparation of this manuscript.

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TABLE 1.—Patient characteristics and treatment results

Characteristic	No. of patients
Total	15
Group I: conventional dose (20 mg/m^2) followed by high-dose MMC (30–50 mg/m^2/course)	8
Group II: high-dose MMC (40 mg/m^2/course)	7
Median age in yrs (range): 52 (29–68)	
Prior radiotherapy	8
Prior hormonal therapy	6
Median No. of prior chemotherapy regimens (range): 2 (1–3)	
Dominant site of disease	
Visceral	9
Soft tissue	5
Bone	1
Overall response	
Complete	0
Partial	1
Minor (less than partial)	5
Hematologic toxicity (40 mg/m^2/course)	
Median neutrophil count nadir (range): 40 cells/μl (0–544)	
Median platelet count nadir (range): 14,000 cells/μl (4000–420,000)	
Hematologic recovery: median No. of days after ABMT (range)	
1st course (40 mg/m^2)	
Neutrophil count > 1000 cells/μl: 20 (16–31)	7/7
Platelet count > 100,000 cells/μl: 32 (19–58)	6/7
2nd course (40 mg/m^2)	
Neutrophil count > 1000 cells/μl: 17 (15–21)	7/7
Platelet count > 100,000 cells/μl: 23 (22–24)	2/7

hepatic veno-occlusive disease, reported previously with high-dose MMC (3,4), was not observed in our study, probably because lower doses were used.

REFERENCES

1. CROOKE ST, and BRADNER WT. Mitomycin C: a review. Cancer Treat Rev 3:121–140, 1976.
2. BUZDAR AU, TASHIMA CK, BLUMENSCHEIN GR, ET AL. Mitomycin C and megestrol acetate in treatment of breast cancer refractory to hormonal therapy and combination chemotherapy. Cancer 41:392–395, 1978.
3. SARNA GP, CHAMPLIN R, WELLS J, ET AL. Phase I study of high-dose mitomycin with autologous bone marrow support. Cancer Treat Rep 66:277–282, 1982.
4. LAZARUS HM, GOTTFRIED MR, HERZIG RH, ET AL. Veno-occlusive disease of the liver after high-dose mitomycin C therapy and autologous bone marrow transplantation. Cancer 49:1789–1795, 1982.

Phase II Evaluation of Vindesine in the Treatment of Malignant Glioma[1,2]

Jane B. Alavi,* Clare B. Weiler, and Leonard A. Bruno[3]

We report a phase II study of vindesine in malignant glioma. This drug was chosen because it is a lipophilic molecule, and thus might be expected to reach poorly vascularized regions of brain tumors. It is also reported to cause less neurotoxicity than vincristine, another vinca alkaloid with some proven activity against brain malignancies (1,2).

Sixteen patients were entered in the study. The patients had a histologic diagnosis of malignant glioma and had previously received radiation therapy and nitrosourea treatment. All patients had progressive disease, as documented by an enlarging tumor on computerized axial tomographic (CT) brain scan, by worsening neurologic status, and by a requirement for corticosteroids to reduce intracranial pressure. The patients had not received radiation therapy for at least 3 months or chemotherapy for at least 6 weeks. Informed consent was obtained. Patient characteristics are shown in table 1.

Vindesine was given weekly at a dose of 3 mg/m^2; after 16 weeks of treatment, the same dose was given every 2 weeks.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total treated	16
Age (yrs)	
Range: 29–62	
Median: 47	
Performance status	
0–1	5
2	7
3	4
Histologic diagnosis	
Malignant glioma	
Grade 3	10
Grade 4	6
Time from diagnosis (mos)	
Range: 4–69	
Median: 9	

Response was defined as improving neurologic status at a stable or decreased corticosteroid dose plus a decrease in tumor size by at least 25% in cross-sectional area on the CT scan. Stable disease was defined as stable tumor size on the CT scan or a < 25% decrease in size, with stable or improving neurologic status, on a stable or decreased corticosteroid dose. This stability had to persist at least 8 weeks from the introduction of vindesine.

All patients were evaluable for response. Two patients responded and the clinical improvement was evident after two doses in each case; they had developed progressive disease while receiving postoperative adjuvant carmustine. For the first patient, headaches and papilledema resolved after vindesine; dexamethasone was discontinued after 4 months. Two and one-half years later, the patient continues to be nearly asymptomatic. Her CT scan showed improvement at 6 months and stabilization thereafter, with a 30% reduction in the enhancing tumor. The other patient demonstrated improvement in hemiparesis and attention span, with a 30% reduction in the tumor on CT scan. The duration of her clinical remission was 11 months.

Five patients had stable disease for 3–28 months (median, 4) by both clinical and CT criteria. Toxic effects were mild (table 2).

DISCUSSION

Other studies of chemotherapy in malignant glioma have shown response rates from 15% to 70% (3,4). However, responses are seen less often in previously treated patients (5) and in those with lower performance status. Median duration of response has been 5–9 months, and median duration of stable disease has been 2–3 months. Our results are similar. We conclude that vindesine may be an active drug for this tumor. A favorable feature of this agent is its low hematologic toxicity at the 3-mg/m^2 dose, even in patients previously treated with nitrosoureas.

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TABLE 2.—Treatment results

	No. of patients
Response	
Partial	2
Stable disease	5
Disease progression	9
Toxic effects	
Leukopenia < 2500 cells/mm^3	4
Thrombocytopenia < 100,000 cells/mm^3	2
Thrombocytopenia < 50,000 cells/mm^3	0
Soft tissue reactions	2
Neurologic	
Paresthesias, mild	4
Constipation	2
Jaw pain	1
Transaminase elevation, transient	2

REFERENCES

1. ROSENSTOCK JG, EVANS AE, and SCHUT L. Response to vincristine of recurrent brain tumors in children. J Neurosurg 45:135-140, 1976.
2. SMART CR, OTTOMAN RE, ROCHLIN DB, ET AL. Clinical experience with vincristine (NSC-67574) in tumors of the central nervous system and other malignant diseases. Cancer Chemother Rep 52:733-741, 1968.
3. EDWARDS MS, LEVIN VA, and WILSON CB. Brain tumor chemotherapy: an evaluation of agents in current use for phase III trials. Cancer Treat Rep 64:1179-1205, 1980.
4. EAGAN RT, DINAPOLI RP, HERMANN RE, JR, ET AL. Combination carmustine (BCNU) and dianhydrogalactitol in the treatment of primary brain tumors recurring after irradiation. Cancer Treat Rep 66:1647-1649, 1982.
5. KESSINGER A, LEMON HM, and FOLEY JF. VM-26 as a second drug in the treatment of brain gliomas. Cancer Treat Rep 63:511-512, 1979.

Phase II Clinical Study of Tamoxifen in Advanced Endometrial Adenocarcinoma: A Gynecologic Oncology Group Study[1,2]

M. Slavik, W. M. Petty, J. A. Blessing, W. T. Creasman, and H. D. Homesley[2,*]

Endometrial adenocarcinoma, one of the most common gynecologic cancers, is known to be responsive to hormonal treatment with progestational agents (1). The presence of estrogen receptors (2-4) has generated interest in clinical studies of antiestrogens in this disease. This paper presents results from a group-wide (Gynecologic Oncology Group [GOG] phase II study of tamoxifen, an antiestrogen of nonsteroidal structure, in 19 patients with advanced or recurrent, histologically confirmed endometrial adenocarcinoma resistant to hormonal treatment with progestational agents and/or chemotherapy. In addition, five patients with histological diagnosis of adenosquamous carcinoma are also included in this report. All patients had measurable disease. Neither ascites nor pleural effusion alone was considered measurable for purposes of this study; sonography was an acceptable measure of disease if the lesion was sharply defined on initial examination. All patients but one had a GOG performance status ≤ 2 (median, 1). Patients must have recovered from nadir blood cell counts from previous therapy and must have had a granulocyte count $\geq 1500/mm^3$ and platelet count $\geq 100{,}000/mm^3$ before study entry. BUN < 25 mg/100 ml, serum creatinine < 1.5 mg/100 ml, bilirubin < 1.0 mg/100 ml, and SGOT < 50 IU/L were required. However, patients with abnormal liver function tests secondary to metastatic tumor were eligible for entry in this study. Standard response criteria were used (5). Estrogen- and progesterone-receptor assays, although desired, were not required.

Tamoxifen in 10-mg tablet form was administered orally at a dose of 10 mg twice a day for a minimum of 8 weeks or until disease progression or adverse effects prohibited further therapy. Patients with stable disease after 8 weeks of therapy had their tamoxifen dose increased to 20 mg twice a day. A course of chemotherapy was defined as 4 weeks.

Patient characteristics, response to therapy, and toxic effects are summarized in table 1. No objective tumor regressions occurred. Of the 19 patients with endometrial adenocarcinoma, 11 had stable disease lasting 8-48 weeks (median, 12) from the start of tamoxifen treatment. Eight patients experienced disease progression after a median of 4 weeks (range, 4-20) of tamoxifen therapy.

Of the five patients with adenosquamous carcinoma of the endometrium, two had stable disease lasting 4-16 weeks and three experienced progressive disease within 2, 4, and 8 weeks of tamoxifen treatment.

Tamoxifen treatment was well-tolerated and only a few patients experienced side effects. Nausea with or without vomiting occurred in four patients and mild leukopenia or anemia occurred in individual patients. Hypercalcemia was reported in one patient during tamoxifen treatment. No flare was reported in any of the patients.

In our experience, tamoxifen given orally at a dose of 10 mg twice a day did not reveal any activity in patients with advanced or recurrent adenocarcinoma or adenosquamous carcinoma of the endometrium, of unknown hormonal-receptor status, progressing after heavy pretreatment with hormonal progestational agents and/or chemotherapy. Additional clinical investigation of the relationship of this drug to estrogen receptors in patients with endometrial adenocarcinoma should be done.

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[2]Supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in the study: University of Kansas Medical Center, Kansas City (*M. Slavik*); University of Oregon Health Sciences Center, Portland (*W. M. Petty*); Roswell Park Memorial Institute, Buffalo, NY (*J. A. Blessing*); Duke University Medical Center, Durham, NC (*W. T. Creasman*, CA-12534); Bowman-Gray School of Medicine of Wake Forest University, Winston-Salem, NC (*H. D. Homesley*, CA-21946); Indiana University Medical Center, Indianapolis (CA-21720); The Milton S. Hershey School of Medicine of Pennsylvania State University, Hershey (CA-16386); University of Alabama in Birmingham (CA-12484); Hahnemann Medical College/Jefferson Medical College, Philadelphia, PA (CA-12478); University of Rochester Medical Center, NY (CA-12482); University of Minnesota Medical School, Minneapolis (CA-23088); Georgetown University Hospital, Washington, DC (CA-16938); University of Texas Health Science Center at Dallas (CA-28160); The Albany Medical College of Union University, NY; University of California Medical Center at Irvine (CA-23765); and Illinois Cancer Council (CA-27806).

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TABLE 1.—Phase II study of tamoxifen in patients with advanced endometrial adenocarcinoma

Age (yrs)	Site of disease*	Performance status	Histology†	Histologic grade	Previous treatment‡	Response to tamoxifen§	No. of courses of tamoxifen¶	Total dose (mg)	Toxic effects and abnormal laboratory values
45	E	2	AC	3	XRT, ADR	ID	2	1220	—
48	E	0	AC	2	XRT, PROVERA, ADR-CTX	SD	3	2020	—
55	P	2	AC	2	XRT, CAP, MEGACE, XRT	SD	4	3220	Nausea, hematocrit (20.7%)
61	E	2	AC	2	CT PROVERA, ADR-CTX	ID	1	960	—
62	P	0	AC	3	XRT-MEGACE	SD	4	2480	—
62	P	1	AC	3	XRT, PROVERA, ADR	SD	3	3460**	—
62	E	2	AC	3	XRT, PROVERA, ADR-CTX	ID	1	760	—
63	E	2	AC	1	XRT, CT PROVERA, CTX-ADR	SD	3	1380	—
64	P	1	AC	1	XRT, PROVERA, ADR	ID	1	520	—
64	E	1	AC	3	ADR-CTX, CT PROVERA-MEGACE	ID	1	600	—
66	E	2	AC	2	XRT, CT PROVERA-ADR	ID	4	2900	Creatinine (1.7 mg/100 ml)
67	P	1	AC	2	CT PROVERA, MEGACE, ADR-CTX	SD	3	2440	Nausea + vomiting, dizziness, BUN (30 mg/100 ml), creatinine (1.7 mg/100 ml)
67	E	1	AC	3	XRT, ADR-CTX, 5-FU, MELPH, PROVERA	SD	3	1760	—
68	E	0	AC	2	MEGACE, XRT, ADR	SD	6	4960	BUN (81 mg/100 ml), SGOT (56 IU/L)
70	E	1	AC	2	XRT, ADR-CTX	ID	5	3320	Nausea
72	P	2	AC	2	CT PROVERA, ADR-CTX	ID	1	760	—
74	E	1	AC	2	CT PROVERA, XRT-MEGACE, ADR	SD	8	8412	—
76	E	1	AC	1	CT PROVERA-MEGACE, ADR	SD	12	11,560	Alkaline phosphatase (137 IU/L), SGOT (36 IU/L)
79	E	2	AC	3	XRT, CTX-5-FU-MEGACE	SD	2	1320	—
45	E	0	AS	2	ADR-CTX-5-FU, MEGACE	ID	1	560	—
55	E	1	AS	2	XRT, MELPH-5-FU	SD	1	620	Upset stomach
56	E	1	AS	2	ADR-CTX-CIS	SD	4	3260	—
63	E	3	AS	3	XRT, MELPH, MTX, VLB	ID	1	260	Hypercalcemia
70	P	2	AS	2	CT PROVERA, ADR	ID	2	1120	Leukopenia (3400 cells/mm^3)

*P = pelvic; E = extrapelvic.

†AC = adenocarcinoma; AS = adenosquamous carcinoma.

‡XRT = irradiation; ADR = doxorubicin; PROVERA = medroxyprogesterone acetate; CTX = cyclophosphamide; CAP = cyclophosphamide, doxorubicin, and cisplatin; MEGACE = megestrol acetate; CT PROVERA = medroxyprogesterone acetate compressed tablets; MELPH = melphalan; CIS = cisplatin; and VLB = vinblastine.

§ID = increasing disease; SD = stable disease.

¶Course was 4 wks.

**Approximate.

REFERENCES

1. DEPPE G. Chemotherapy treatment of endometrial carcinoma. Clin Obstet Gynecol 25:93-99, 1982.
2. TERENIUS L, LINDELL A, and PERSSON BH. Binding of estradiol-17 to human cancer tissue of the female genital tracts. Cancer Res 31:1895-1898, 1979.
3. GURPIDE E. Hormone receptors in endometrial cancer. Cancer (Suppl) 48:638-641, 1981.
4. MORTEL R, LEVY C, WOLFF JP, ET AL. Female sex steroid receptors in postmenopausal endometrial carcinoma and biochemical response to an antiestrogen. Cancer Res 41:1140-1147, 1981.
5. THIGPEN JT, BUCHSBAUM HJ, MANGAN C, ET AL. Phase II trial of adriamycin in the treatment of advanced or recurrent endometrial carcinoma: a Gynecologic Oncology Group study. Cancer Treat Rep 63:21-27, 1979.

Treatment of Metastatic Sarcomas With Mitoxantrone[1,2]

Cary A. Presant,[3,*] Richard Gams,[4] Alfred A. Bartolucci,[4] and the Southeastern Cancer Study Group

The treatment of metastatic sarcomas with chemotherapy has focused on the use of doxorubicin and doxorubicin-containing regimens (1). To identify new active agents, mitoxantrone, a new anthracene derivative which has demonstrated good antitumor activity against samples of human soft tissue sarcomas in a human tumor colony-forming system (2), was tested in a phase II trial.

METHODS

Patients were eligible for protocol 80 SAR 281 if they had soft tissue or bone sarcoma incurable by standard techniques, had measurable tumor, had no prior doxorubicin or uncompensated cardiac disease, had given voluntary informed consent, and had a performance status of $\geq 40\%$. Mitoxantrone (5 mg/m^2) was given iv over 30 minutes weekly until tumor progression. Treatments were delayed until granulocyte counts were $> 1500/mm^3$ and platelet counts were $> 90{,}000/mm^3$. Doses were decreased for myelosuppression, severe vomiting, or hyperbilirubinemia. A partial response (PR) was defined as a $> 50\%$ reduction in the sum of tumor areas, and disease progression was defined as a $> 25\%$ increase.

RESULTS

Of the 43 patients with sarcomas resistant to prior chemotherapy (table 1), four were inevaluable (no data or wrong doses); no patient achieved a PR. Of the 18 patients with sarcomas not resistant to prior chemotherapy, two had not been in the study long enough for final analysis and two were inevaluable (did not have sarcoma or received only one course); no patient achieved a PR.

The dose-limiting toxic effect was granulocytopenia (table 1). Thrombocytopenia was observed in only 5% of patients with previously treated sarcomas. Severe vomiting was unusual. Ten percent of the patients with previously treated sarcomas had an unexplained increase in serum creatinine concentration to less than twice normal. Although two patients had decreased voltage on ECG, no patient had congestive heart failure.

DISCUSSION

Mitoxantrone has undergone considerable phase I and II testing (3-5). Although the drug has antitumor activity against several tumors, we were disappointed that there was no activity against metastatic sarcomas in our trial.

Considerable interest has been expressed in the use of human tumor colony-forming assays in vitro to predict which drugs should be tested in phase II trials. Although the investigators in such studies do not predict direct responses with certainty, it was disappointing to observe no clinical activity whatsoever of mitoxantrone in sarcomas, despite good antitumor activity against sarcomas in colony-forming assays (2). This cautions against extreme reliance on colony-forming assays to select drugs to be used clinically in phase II trials.

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TABLE 1.—Mitoxantrone in sarcomas: patient characteristics and response*

	Resistant to prior chemotherapy	No prior chemotherapy
Entered	43	18
Evaluable	39	14
Sex		
Male	25	6
Female	14	8
Median age (yrs)	52	50
Tumor histology		
Liposarcoma	4	0
Malignant fibrous histiocytoma	5	0
Fibrosarcoma	3	3
Leiomyosarcoma	15	3
Osteosarcoma	6	1
Other	6	7
Pretreatment performance status		
80–100	23	9
50–70	13	5
40–50	3	0
Response		
PR	0	0
Stable disease	10	6
Disease progression	29	8
Moderate or severe toxicity (% of patients)		
Granuiocytopenia < 1500 cells/mm^3	50	46
Thrombocytopenia < 90,000 cells/mm^3	5	0
Hyperbilirubinemia	2	0
Vomiting	13	0

* Unless otherwise specified, values = No. of patients.

REFERENCES

1. PRESANT CA, LOWENBRAUN S, BARTOLUCCI AA, ET AL. Metastatic sarcomas: chemotherapy with adriamycin, cyclophosphamide, and methotrexate alternating with actinomycin D, DTIC and vincristine. Cancer 47:457–465, 1981.
2. VON HOFF DD, COLTMAN CA, and FORSETH B. Activity of mitoxantrone in a human tumor cloning system. Cancer Res 41:1853–1855, 1981.
3. STEPHENS RL, and CLARK G. Southwest Oncology Group (SWOG) phase II study with mitoxantrone in non-hematologic malignancies. *In* Proceedings of the Third NCI-EORTC Symposium on New Drugs in Cancer Therapy, Brussels, Oct 15–17, 1981. Brussels, European Organization for Research on Treatment of Cancer, 1981, p A36.
4. YAP HY, BLUMENSCHEIN GR, SCHELL FC, ET AL. Dihydroxyanthracenedione: a promising new drug in the treatment of metastatic breast cancer. Ann Intern Med 95:694–697, 1981.
5. GAMS RA, TERZAKIS TA, and BARTOLUCCI AA. Phase II studies of mitoxantrone. *In* Proceedings of the Third NCI-EORTC Symposium on New Drugs in Cancer Therapy, Brussels, Oct 15–17, 1981. Brussels, European Organization for Research on Treatment of Cancer, 1981, p A38.

Teniposide in Refractory Ovarian Cancer: A Phase II Study[1]

Cristiana Sessa,[2] Maurizio D'Incalci,[2,*] Fabio Landoni,[3] Nicoletta Colombo,[3] and Costantino Mangioni[3]

Ovarian cancer refractory to conventional treatment reportedly responded occasionally to single intermittent doses of the podophyllotoxin derivative teniposide (VM-26) (1,2). However, no objective responses were seen in 16 patients with ovarian cancer receiving 30 mg/m²/daily × 5 every 3-4 weeks (3). The suggestion of a dose-response relationship in small cell lung cancer (4) prompted us to further investigate the activity of weekly increasing doses of VM-26. Since VM-26 toxicity seems to be influenced by the exposure time of cancer cells (5), we tested the same dose administered by 1- or 24-hour continuous infusion.

PATIENTS AND METHODS

Twenty-three patients with ovarian cancer were entered in this study. They were 22-70 years of age, had a performance status ≥ 70, and had measurable progressive disease after conventional therapy. All patients had received at least cisplatin and one alkylating agent, alone or in combination; previous treatment included doxorubicin (14 patients) and radiotherapy (nine).

Patients received a starting dose of 100 mg/m² by 1-hour infusion (first course). On Day 8, if the wbc count was ≥ 4000/μl and/or the platelet count was ≥ 100,000/μl, patients received 150 mg/m² by 1-hour infusion (second course). On Day 22, if the wbc count was ≥ 4000/μl and/or the platelet count was ≥ 100,000/μl, patients received 150 mg/m² by 24-28-hour infusion (third course). If the wbc count was < 4000/μl and/or the platelet count was < 100,000/μl, treatment was postponed 1 week. If the wbc count was 3000-4000/μl and/or the platelet count was 75,000-100,000/μl after 1 week, VM-26 was then administered at 100 mg/m². Treatment was deferred for another week if the wbc count was < 3000/μl and/or the platelet count was < 75,000/μl. Treatment was stopped if a wbc count of < 3000/μl and/or a platelet count of < 75,000/μl persisted 3 weeks after the previous dose.

Patients were evaluable for toxic effects after one course and for response after two courses. Responses were defined according to previously described criteria (6). Patients showing objective response or stable disease received 150 mg/m² by 24-hour infusion every 2-3 weeks until disease progression.

RESULTS

Of the 23 patients, 20 were evaluable for response and 22 for toxic effects; three patients were excluded from analysis for refusal of therapy, inadequate follow-up data, and persistent leukopenia after the first course. Sixteen of the 20 evaluable patients received at least three courses of therapy, including one prolonged infusion, whereas treatment was stopped after the second course because of disease progression in three patients and persistent leukopenia in one.

No patient achieved a partial response. Three patients had stable disease lasting 6, 3, and 4 months.

Table 1 shows the median wbc and platelet counts 8 and 15 days after different doses and times of infusion. Leukopenia was the main side effect, requiring a 1-week postponement in four patients after 100 mg/m² and in nine after 150 mg/m² by 1-hour infusion. Doses were increased in all but one patient (previously irradiated, given 100 mg/m² for all three courses). Nausea and controllable vomiting occurred in 55% of patients after the second course and in 25% after the third; cumulative alopecia was reported in 31%.

DISCUSSION

When administered on a weekly schedule, relatively high doses of VM-26 given by 1- or 24-hour infusion do not appear to be effective in refractory ovarian cancer. The schedule dependency of the antitumor effect of the epipodophyllotoxins in animals (7) and the results achieved in some human malignancies with etoposide (8),

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TABLE 1.—Median values and range of wbc and platelet counts in relation to VM-26 treatment

Dose (mg/m^2)	Duration of infusion (hrs)	Wbc count* after—		Platelet count* after—	
		8 days	15 days	8 days	15 days
		All patients			
100	1	5.0 (2.6-7.0)	—	186 (105-403)	—
150	1	3.7 (0.9-7.3)	3.4 (2.3-7.2)	145 (90-405)	220 (61-490)
150	24-28	4.4 (1.7-6.2)	4.9 (1.5-6.8)	223 (152-520)	182 (92-468)
		Patients with previous radiotherapy			
100	1	3.9 (2.6-6.1)	—	170 (150-403)	—
150	1	3.8 (0.9-7.3)	3.1 (2.3-6.8)	123 (91-230)	224 (61-300)
150	24-28	4.9 (1.7-6.2)	5.2 (1.5-6.8)	262 (194-520)	216 (137-468)
		Patients without previous radiotherapy			
100	1	5.4 (3.4-7.0)	—	186 (132-390)	—
150	1	3.7 (1.4-4.8)	3.8 (3.2-7.2)	170 (90-405)	210 (119-490)
150	24-28	4.2 (3.1-5.0)	4.6 (1.8-6.4)	226 (178-378)	172 (92-367)

*$\times 10^3$ cells/ml. Values = median (range).

however, also suggest that VM-26, also, could be more effective when given as daily repeated doses or infusions lasting > 24 hours. Until now, there have been no controlled studies comparing toxicity or efficacy of the different schedules. Treatment was very well tolerated, considering that all patients had previously received heavy chemotherapy and about 44% had also received radiotherapy. A dose of 150 mg/m^2 given by 24-hour infusion caused much less nausea and vomiting, with slightly milder hematologic toxicity, than the same dose given by 1-hour infusion. As previously reported (9), continuous infusions of different dose ranges could therefore be safely evaluated.

REFERENCES

1. SPREMULLI E, SCHULZ JJ, SPECKHART VJ, ET AL. Phase II study of VM-26 in adult malignancies. Cancer Treat Rep 64:147-149, 1980.
2. JANKOWSKI RP, and VAHRSON H. Die Toxizität hoher Dosen von VM26 (4-demethyl-epipodophyllotoxin-β-D-thenylidene glucoside). Ein Beitrag zur Behandlung des fortgeschrittenen Ovarialkarzinoms. Med Klin 72:2122-2126, 1977.
3. SAMSON MK, BAKER LH, TALLEY RW, ET AL. VM26 (NSC-122819): a clinical study in advanced cancer of the lung and ovary. Eur J Cancer 14:1395-1399, 1978.
4. WOODS RL, FOX RM, and TATTERSALL MHN. Treatment of small cell bronchogenic carcinoma with VM-26. Cancer Treat Rep 63:2011-2013, 1979.
5. HILL BT, WHELAN RDH, RUPNIAK HT, ET AL. A comparative assessment of the in vitro effects of drugs on cells by means of colony assays or flow microfluorimetry. Cancer Chemother Pharmacol 7:21-26, 1981.
6. SESSA C, D'INCALCI M, VALENTE I, ET AL. Hexamethylmelamine-CAF (cyclophosphamide, methotrexate, and 5-FU) and cisplatin-CAF in refractory ovarian cancer. Cancer Treat Rep 66:1233-1234, 1982.
7. DOMBERNOWSKY P, and NISSEN NI. Schedule dependency of the antileukemic activity of the podophyllotoxin-derivative VP 16-213 (NSC 141540) in L1210 leukemia. Acta Pathol Microbiol Scand [A] 81:715-724, 1973.
8. CAVALLI F, SONNTAG RW, JUNGI F, ET AL. VP-16-213 monotherapy for remission induction of small cell lung cancer: a randomized trial using three dosage schedules. Cancer Treat Rep 62:473-475, 1978.
9. BOOSER DJ, SPITZER G, CHIUTEN DF, ET AL. Epipodophyllotoxins VP16-213 and VM26: a phase I study of 5-day continuous intravenous infusion (CI) in bronchogenic carcinoma. Proc Am Assoc Cancer Res and ASCO 22:508, 1981.

Warfarin, Etoposide, and Vindesine Interactions

Drug interactions with warfarin sodium have been well-documented (1). To date, no such interactions have been reported with antineoplastic drugs. We report in this letter a probable drug interaction between warfarin, vindesine, and etoposide.

A 78-year-old man with adenocarcinoma of the lung (stage III_{M1}, bone metastases) was referred to the Michael Reese Medical Center for treatment evaluation. During the course of his hospitalization, he had an episode of thrombophlebitis and was treated with heparin and warfarin. Because of the widespread nature of the adenocarcinoma of the lung with metastases to bone, pleura, and chest wall, chemotherapy was recommended and the patient agreed to participate in an experimental program of vindesine, etoposide, and cisplatin (VEP). Table 1 shows his daily prothrombin time and warfarin dose relative to the administration of VEP.

The warfarin dose was stable on Days 19-22 and 30-37. However, despite a stable maintenance dose of warfarin, an abrupt elevation of the patient's prothrombin time occurred with the administration of vindesine and etoposide on Days 15, 22, and 37. The VEP regimen was discontinued after the 42nd day due to progressive adenocarcinoma. Warfarin was resumed at a 2.5-mg dose on Day 44. The patient was maintained on warfarin (2.5 mg/day) until his death on Day 119.

Etoposide and vindesine are both mitotic spindle inhibitors (2,3), with activity against a variety of solid tumors. Both drugs cause myelosuppression that is dose-related and not cumulative. Etoposide is bound to serum proteins, while vindesine is not. Warfarin is absorbed in the small intestine and is bound to albumin within the bloodstream (1). Within the liver, warfarin is biotransformed by enzymes located on the endoplasmic reticulum. Furthermore, the metabolites of warfarin are also bound to albumin before excretion in the urine and bile (1). Several opportunities for drug interactions between warfarin and etoposide or vindesine can be postulated. Etoposide may appreciably prolong the prothrombin time and enhance the anticoagulant effect by displacing warfarin from binding sites on albumin. Whether etoposide or vindesine or their individual metabolites slow the rate of warfarin biotransformation by effects on the requisite degradative enzymes or compete for sites of biotransformation on the endoplasmic reticulum is unknown at this time. Additionally, deleterious effects on vitamin K metabolism, as well as intake, may be coincidental with the gastrointestinal toxic effects of these two drugs.

In view of the possible interaction of warfarin and etoposide or vindesine, and the possible benefit of warfarin in reducing tumor growth (4), close observation of patients receiving warfarin and either etoposide or vindesine is recommended.

REFERENCES

1. KOCH-WESER J, and SELLERS EM. Drug interactions with coumadin anticoagulants. N Engl J Med 285:478-498, 547-558, 1971.
2. VOGELZANG NJ, RAGHAVEN D, and KENNEDY BJ. The mandrake root from Issyk-Kul. Am J Med 72:136-144, 1982.
3. DYKE RW, and NELSON RL. Phase I anticancer agent, vindesine (desacetyl vinblastine amide sulfate). Cancer Treat Rev 4:135, 1977.
4. ZUCHARCKI LR, HENDERSON WG, RICKLES FR, ET AL. Rationale and

TABLE 1.—Prothrombin time and warfarin dose related to the administration of VEP

Day	Warfarin dose (mg)	Prothrombin time (secs)	Vindesine (mg)	Etoposide (mg)	Cisplatin (mg)
6	15	10	—	—	—
7	15	23	5	500	200
8, 9	—	24	—	—	—
10, 11	10	18.0	—	—	—
12-14	5	20.8	—	—	—
15	5	21.3	5	500	—
16-18	—	29, 29, 23	—	—	—
19-21	2.5	21.3	—	—	—
22	2.5	21.0	5	500	—
23-25	—	30, 32, 30	—	—	—
30-36	2.5	19.5	—	—	—
37	2.5	20.7	5	500	200
38-40	—	31.3, 35.4, 30.3	—	—	—

experimental design for the VA cooperative study of anticoagulation (warfarin) in the treatment of cancer. Cancer 44:732–741, 1979.

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December 29, 1983

Subcutaneous Reaction Due to Intra-arterial Infusion of Anthracycline

The reaction described by Ballin et al (1) is simply part of a spectrum of complications which may occur with intra-arterial doxorubicin (2,3). These comprise erythema, toxic epidermal injury, epidermolysis, induration, necrosis, and finally, ulceration. The cause is undetermined, although high concentrations of a vesicant drug have been reported to cause inflammatory and necrotic reactions in perfused tissues (4). This may be aggravated by spasm or irritation of small vessels. It has been claimed that skin reactions may be avoided by positioning the catheter in a vessel with a large caliber bore (D. Morton, personal communication). However, entry of the chemotherapeutic agent into arterial tributaries may be occasioned by "streaming" and not necessarily by the direction of the catheter tip. Extravasation as a possible cause has been excluded by the absence of fluorescence on Wood's lamp examination (3).

Skin reactions have also been encountered with intra-arterial cisplatin (CDP). These reactions have developed in > 90% of the infusions and are manifested as localized areas of pigmentation and induration in close proximity to the tip of the catheter (5). In one case, biopsy revealed tissue destruction and increased concentrations of CDP. Whether this occurred by direct deposition of the drug or by "seepage" from surrounding high concentrations is uncertain. The high local tissue content may also have been related to vascular damage, compromising drug elimination. The detection of the high CDP content suggests that the offending mechanism be different to the doxorubicin phenomenon. We have been unable to prevent the complication with intra-arterial or iv hydrocortisone, although amelioration may have been achieved in some cases.

In contrast to intra-arterial doxorubicin, ulceration is rare with CDP. Since localized sloughing and ulceration may interfere with planned operative procedures, we have chosen to use CDP rather than doxorubicin alone or in combination for limb salvage candidates.

REFERENCES

1. BALLIN A, KENDE G, MORAG B, ET AL. Subcutaneous reaction due to intra-arterial infusion of doxorubicin. Cancer Treat Rep 66:2000–2001, 1982.
2. VON EYBEN FE, BRUZE M, EKSBORG S, ET AL. Toxic epidermal injury following intra-arterial adriamycin treatment. Cancer 48:1535–1538, 1981.
3. JAFFE N, WATTS H, FELLOWS KE, ET AL. Local en bloc resection for limb preservation. Cancer Treat Rep 62:217–223, 1978.
4. SHAH P, BAKER LH, and VAITKEVICIUS VK. Preliminary experiences with intra-arterial adriamycin. Cancer Treat Rep 61:1565–1567, 1977.
5. JAFFE N, KNAPP J, CHUANG VP, ET AL. Osteosarcoma: intra-arterial treatment of the primary tumor with *cis*-diamminedichloroplatinum II (CDP). Angiographic, pathologic and pharmacologic studies. Cancer 51:402–407, 1983.

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December 29, 1983

Complete Remission in Refractory Burkitt's Lymphoma

In American trials, Burkitt's lymphoma is curable in 50%–70% of cases (1). Ultimately, 30%–50% of patients will be candidates for second-line therapy. The best salvage regimen, carmustine, cytarabine, cyclophosphamide, and thioguanine (BACT), is associated with only about a 30% long-term disease-free status (2). There are no known long-term survivors who have failed BACT; most patients die in a short period of time. This case report discusses a patient with refractory Burkitt's lymphoma who achieved a complete remission.

CASE REPORT

A 33-year-old white male was diagnosed as having Burkitt's lymphoma in March 1982, with disease in the abdomen and bone. He received six cycles of cyclophosphamide, vincristine, and methotrexate through July 1982 and achieved a disease-free status. In August 1982, a gallium scan suggested recurrent Burkitt's lymphoma. Bilateral bone marrow biopsies were negative. The patient was treated with BACT without autologous marrow

transplant in September 1982. Again, he was rendered disease-free. In November 1982, he developed pain in his right leg. X-rays showed a new lucency in that area, and bone scan revealed increased uptake in the right femur. LDH was > 2000 units/L. The patient was then treated with four cycles of cisplatin, vinblastine, bleomycin, and doxorubicin (PVBA) (3) through February 1983. He developed marked pancytopenia due to the chemotherapy. After the fourth cycle, he developed klebsiella sepsis, acute renal failure, and adult respiratory distress syndrome and died in March 1983. At autopsy, which included multiple bone biopsies in areas of previously known disease, there was no evidence of lymphoma.

DISCUSSION

PVBA is a regimen that is highly effective in testicular cancer (3), a cancer with a high growth fraction similar to that of Burkitt's lymphoma. In addition, the four drugs used are all active in lymphoma. It would seem logical to assume that this regimen may be efficacious in Burkitt's lymphoma.

It is impossible to predict whether the response that this patient had would have been a durable complete remission. It does appear that this cisplatin-based combination chemotherapy regimen is active in refractory Burkitt's lymphoma. The prognosis is so dismal in these patients that further study with PVBA may be worthwhile pursuing in refractory patients or possibly incorporated as a non-cross-resistant regimen in initial therapy.

REFERENCES

1. ZIEGLER JL. Burkitt's lymphoma. N Engl J Med 305:735–745, 1981.
2. APPLEBAUM FR, DEISSEROTH AB, GRAW RG, JR, ET AL. Prolonged complete remission following high dose chemotherapy of Burkitt's lymphoma in relapse. Cancer 41:1059–1063, 1978.
3. EINHORN LH, and WILLIAMS SD. Chemotherapy of disseminated testicular cancer: a random prospective study. Cancer 46:1339–1344, 1980.

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January 12, 1984

Position Vacancy: Head, Quality Assurance and Compliance Section

The position of Head, Quality Assurance and Compliance Section is now vacant. This position is located within the Cancer Therapy Evaluation Program of the National Cancer Institute (NCI), National Institutes of Health, Department of Health and Human Services.

This is a Civil Service career-conditional appointment under the Merit Pay system at the GM-14 level, with a salary range from $49,533 to $61,917 per year depending upon the qualifications of the applicant. Candidates may be eligible for Physicians' Comparability Pay up to $10,000 per year.

The Head of the Quality Assurance and Compliance Section has the overall responsibility for managing the site-visit monitoring process for all NCI clinical trials using NCI-sponsored investigational drugs and biological response modifiers. Applicants should be Board eligible or certified Oncologists.

Applications (personal qualifications statement SF-171) are to be sent to the address below. A current curriculum vitae and bibliography must accompany all applications. The National Institutes of Health is an equal opportunity employer.

For further information, contact: Cynthia A. Kauff, PMS, National Cancer Institute, Bldg 31, Rm 3A08, Bethesda, MD 20205 (telephone: 301-496-6503).

Koloman Laki Memorial Symposium on Haemostasis and Cancer

Budapest, Hungary, July 9–11, 1984

In memory of the late Dr. Koloman Laki, codiscoverer of Factor XIII and author of many highly valuable contributions to the fields of haemostasis and cancer, an International Symposium on Haemostasis and Cancer, jointly organized by the National Foundation for Cancer Research, and the Hungarian Society on Haematology, Haemostasis Section, will be held at the Hotel Hyatt Atrium in Budapest.

The symposium is open to scientists interested in various aspects of the relationship of haemostatic systems and malignant cell proliferation and metastasis. Lectures will focus on procoagulant materials produced by cancer cells, the role of fibrinogen-fibrin-Factor XIII in cancer cell proliferation, the effect of anticoagulant and antiplatelet drugs on malignant tumors, and on platelet-malignant cell interaction. Additionally, there will be posters and oral presentations. The number of the participants is limited to 100. The Congress language will be English.

For further information, contact: Dr. L. Muszbek, University School of Medicine, Department of Clinical Chemistry, PO Box 40, Debrecen Hungary H-4012 (telephone: /36/-52-17631, telex: 72-411 dote-h).

Epidemiology Summer Program

Chestnut Hill, Massachusetts, July 29–August 17, 1984

The New England Epidemiology Institute announces its summer program which will be hosted by the University of Massachusetts, and take place at the Amherst Campus in western Massachusetts. The program includes both methodologic and substantive courses, and will have classes for those seeking an introduction to modern epidemiologic concepts as well as those desiring a review of recent developments in epidemiologic thinking. Tentatively, 14 courses will be offered, including Epidemiologic Methods (introductory and advanced levels), Introductory Biostatistics, Intermediate Biostatistics, Modeling for Epidemiologic Research (Logistic Regression and Cox Survival), Cancer Epidemiology, Cardiovascular Disease Epidemiology, Nutritional Epidemiology, the Epidemiology of Reproductive and Congenital Disorders, Infectious Disease Epidemiology, Environmental and Occupational Epidemiology, Applications of Epidemiology to Health Services, Microcomputers in Epidemiologic Research, and Biomedical Writing. Invited faculty include excellent teachers and prominent researchers from leading universities. Registrants may receive credits toward graduate degrees, continuing medical education, or certification maintenance in industrial hygiene.

For more information, contact: New England Epidemiology Institute, Department SC-4, PO Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

Fellowship in Medical Oncology, Hematology, or Combined Oncology-Hematology

July 1984 and July 1985

Applications are now being accepted for a fellowship program involving major teaching hospitals of Brown University's Program in Medicine, beginning July 1984 and July 1985. Clinical activities and opportunities for research are offered to qualified candidates with two years of approved medical residency and ABIM eligibility.

For further information, contact: F. J. Cummings, MD, Director, Division of Medical Oncology, or Manfred Steiner, MD, Director, Division of Hematology, Brown University, Box G, Providence, RI 02912.

Tenth International RES Congress

Ito, Japan, September 2–7, 1984

The scientific program of this meeting to be held at the Kawana Hotel, will be chaired by Drs. Dolph O. Adams and Ralph Snyderman (Duke University, Durham, NC). Dr. Mizu Kojima (University of Tsukuba, Ibaraki-ken, Japan) is the President of the Congress and International Host.

The scientific sessions will consist of symposia, mini-symposia, targeted sessions, poster sessions, and a keynote address. Symposium topics include: Viruses, Oncogenes and Human Lymphomas; Macrophages and Atherogenesis; Macrophages as Regulators of Multiple Host Systems; Macrophages and Activation; and Macrophages and Stimulus-Response Coupling.

For further information, contact: Reticuloendothelial Society, c/o Dr. Sherwood M. Reichard, Medical College of Georgia, Augusta, GA 30912 (404-828-2601).

Conference on Immunity to Cancer

Williamsburg, Virginia, September 10–12, 1984

A conference to present advances in the various branches of cancer immunology will be held at the Colonial Williamsburg Conference Center. This conference is co-sponsored by the Biological Response Modifiers Program (BRMP) of the National Cancer Institute and the Monsanto Chemical Company. Other sponsors are the Hoffmann-La Roche Co, Eli Lilly and Co, Johnson & Johnson, Inc, New England Nuclear-DuPont, Inc, Bristol-Myers, Inc, ICI-Americas, and McNeil Pharmaceutical. Co-chairmen are Drs. Arnold E. Reif and Malcolm S. Mitchell.

Five sessions of invited papers will be presented entitled: Identification and Characterization of Tumor Antigens; Immune Responses to Tumor Antigens; Regulation of Immune Responses to Tumor Cells; Immunotherapy and Biomodulators; and Immunotherapy and Future Prospects. Participants are invited to submit a one-page abstract, identified by session title, for a Poster Session (deadline: *June 15, 1984*). From the abstracts chosen for the poster presentation, a minority will be selected for inclusion in the Proceedings.

There will be a registration fee of $75 made payable to Program Resources, Inc (deadline: *July 15, 1984*). Early registration is suggested as the conference is limited to 300 participants.

For further information, contact: Ms. Carole Kirby, BRMP, Division of Cancer Treatment, National Cancer Institute, Frederick Cancer Research Facility, Bldg 567, Rm 129, Frederick, MD 21701 (301-695-1418).

Urologic Cancer Course

Boston, Massachusetts, October 1–3, 1984

This intensive, 3-day course, to be held at the Parker House Hotel, will provide an overview of the clinical and pathophysiologic basis for the diagnosis and treatment of patients with urologic cancer. This course will emphasize the multidisciplinary approach which constitutes the foundation of contemporary care for patients with urologic cancers. Principles derived from the disciplines of urologic surgery, medical oncology, and radiation therapy will be highlighted and incorporated into a comprehensive consideration of therapeutic practices. While teaching will be primarily provided through lectures, panel discussion of problem management cases will be an integral part of the course. The course will

emphasize cancers of the prostate gland, kidney, bladder, and testis. A comprehensive syllabus will be provided.

Guest faculty will include: Drs. Lawrence H. Einhorn, Carl A. Olsson, Michael J. Peckham, and Patrick C. Walsh. The Harvard/Boston faculty includes leaders in urologic cancer from the Dana-Farber Cancer Institute, Brigham and Women's Hospital, Beth Israel Hospital, Massachusetts General Hospital, Joint Center for Radiation Therapy, Lahey Clinic, University Hospital, Boston, City Hospital, and Children's Hospital Medical Center.

The course fee is $350, with a reduced rate of $200 for residents, house staff, fellows, and nurses.

For further information, contact: Harvard Medical School, Department of Continuing Education, Boston, MA (617-732-1525).

Twenty-First National Reticuloendothelial Society Meeting

Montreal, Canada, October 14–17, 1984

The Scientific Program Chair of this meeting to be held at the Meridien Hotel, is Dr. Dolph O. Adams (Duke University, Durham, NC) and the Local Host Chair is Dr. Emil Skamene (Montreal General Hospital, Canada).

The scientific sessions will consist of a discussion workshop, symposia, mini-symposia, targeted sessions, poster sessions, and a keynote address. Symposium topics include: Cell-Cell Interaction: A Basic Host Recognition System; Recent Advances in Neutrophil Biology; Human Macrophages and Disease, and Cellular and Molecular Biology of Interferon.

For further information, contact: Reticuloendothelial Society, c/o Dr. Sherwood M. Reichard, Medical College of Georgia, Augusta, GA 30912 (404-828-2601).

International Workshop on the Use of Rodent Tumors in Experimental Cancer Therapy

October 21–24, 1984

This workshop is strictly limited to 60 investigators active in experimental radiotherapy, chemotherapy, and immunotherapy. This workshop will attempt to reach agreement on practices to follow when working with tumors in laboratory animals. The agenda will cover the following topics: Assays of therapeutic effectiveness by tumor control (TCD_{50}), tumor regrowth, and excision assay (both in vivo and in vitro); Choice of suitable tumor lines and their maintenance; Suitable host animal strains; Suitability of xenografts; and Statistical principles and details.

Plans are being made to hold the meeting outside the US, but the final location has not yet been determined. However, travel and attendance will be grant-supported. Those interested in attending should apply by *June 15, 1984* including a letter summarizing your reasons for wishing to attend, your experience in this field, your present position and location, and a list of up to five of your publications that are relevant.

For further information, contact: Dr. R. F. Kallman, Department of Radiology, Stanford University Medical School, Stanford, CA 94305.

Cincinnati Cancer Conference III

Cincinnati, Ohio, November 2–3, 1984

Lung cancer is the focus of this conference to be held at the Hyatt Regency in Cincinnati.

Speakers for the conference are Paul A. Bunn, MD, Lawrence Einhorn, MD, Harvey M. Golomb, MD, George M. Hahn, PhD, Heber MacMahon, MD, Mary J. Matthews, MD, Clifton Mountain, MD, Carlos Perez, MD, Stephen Stellman, PhD, and Ronald Stephens, MD.

The course director is Barth Hoogstraten, MD, and Bethesda Hospitals will sponsor the event.

For further information, contact: Thomas J. O'Connor, Medical Staff Education, Bethesda Hospitals, 619 Oak St, Cincinnati, OH 45206 (1-513-569-6337).

Third International Congress on Hormones and Cancer

Rome, Italy, April 23-27, 1986

For information, contact: Stefano Iacobelli, MD, Laboratorio di Endocrinologia Molecolare, Istituto di Clinica Ostetrica e Ginecologica, Universita Cattolica del S. Cuore, L.go Agostino Gemelli, 8, 00168-Rome, Italy (telephone: (06) 330.54.496-33.70.551, telex: 611330 UNCATRO).

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